Prostaglandin E₂ Regulates Cell Migration via the Intracellular Activation of the Epidermal Growth Factor Receptor*

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Over the past decade cyclooxygenase-2-derived prostaglandins have been implicated in the development and progression of many types of cancer. Recently our laboratory has shown that treatment with prostaglandin E₂ (PGE₂) induces increased proliferation, migration, and invasiveness of colorectal carcinoma cells (Sheng, H., Shao, J., Washington, M. K., and DuBois, R. N. (2001) J. Biol. Chem. 276, 18075–18081). The stimulatory effects of PGE₂ were dependent upon the activation of the phosphatidylinositol 3-kinase/Akt pathway. However, the exact signaling cascade responsible for phosphatidylinositol 3-kinase/Akt activation by PGE₂ remains poorly defined. In the present study, we demonstrate that the PGE₂-induced migration and invasion occurs via rapid transactivation and phosphorylation of the epidermal growth factor receptor (EGFR). Within minutes following treatment, PGE₂ induces the activation of Akt. This effect was completely abolished by EGFR-specific tyrosine kinase inhibitors providing evidence for the role of the EGFR in this response. The rapid transactivation of the EGFR occurs via an intracellular Src-mediated event but not through the release of an extracellular epidermal growth factor-like ligand. EGFR transactivation was also observed in vivo by the direct comparison of normal and malignant human colorectal samples. These results suggest that in developing colonic carcinomas, the early effects of cyclooxygenase-2-derived PGE₂ are in part mediated by the EGFR, and this transactivation is responsible for subsequent downstream effects including the stimulation of cell migration and invasion.

Over the last few years, a heightened interest in the role of COX-2 in neoplasia has emerged. This interest originally arose from epidemiological studies showing up to a 50% reduction in the mortality rate from certain cancers in patients consuming nonsteroidal anti-inflammatory drugs (NSAIDS), such as aspirin (1–3). Because of these initial findings, the use of NSAIDS and COX-2 selective inhibitors has been shown to inhibit growth and metastasis in many cell cultures and animal models of a variety of malignancies (4–8). These inhibitors have also been known to inhibit angiogenesis (7, 9, 10). Transient expression of COX-2 alone can stimulate proliferation and invasion of colorectal carcinoma cells (11, 12). When COX-2 knockout mice are crossed with mice that are heterozygous for an APC<sup>−/−</sup> mutation, the resulting progeny had a substantial reduction of intestinal tumors (13). Furthermore, overexpression of COX-2 in breast tissue in transgenic mice leads to the development of breast carcinomas, indicating that it plays a key role in tumor promotion in certain contexts (14). However, the definitive mechanism(s) through which COX-2 mediates these effects remains unknown.

Cyclooxygenase (or prostaglandin endoperoxide synthase) catalyzes the conversion of arachidonic acid to PGH₂. This unstable endoperoxide is then converted into PGE₃, PGI₂, PGP₂, PFP₂, or thromboxane A₂ by each respective PG synthase. Most tumors that express cyclooxygenase have been found to contain high levels of PGE₂ and the microsomal PGE synthase enzyme. Presumably, these bioactive lipid products of cyclooxygenase, such as PGE₂, are responsible for some of the pro-neoplastic effects mediated by this enzyme. Currently, there are two known isoforms of cyclooxygenase, which are referred to as COX-1 and COX-2. COX-1 is expressed constitutively in many tissues; however, recent evidence (15, 16) indicates that the expression of COX-1 is induced in some tumors, such as ovarian cancer. Conversely, COX-2 is not expressed constitutively in many situations, although it can be induced in the normal brain, kidney, and reproductive tract. COX-2 expression is induced by many growth factors, cytokines, and tumor promoters (17), and elevated expression of COX-2 has been observed in many malignancies including the breast, lung, and colon. The prostaglandin products of COX-1 and COX-2 have been shown to exert many biological properties such as the induction of inflammation, the maintenance of kidney function, and the modulation of the immune response (18). Our laboratory has shown recently that COX-derived PGE₂ can induce the growth, migration, and invasiveness of colorectal carcinoma cells (19). PGE₂ also induced a change in morphology of these cells by stimulating the reorganization of actin stress fibers and focal adhesion complexes. In these studies, PGE₂-induced the activation of PI 3-kinase and its immediate downstream effector Akt (or protein kinase B). We found that inhibition of PI 3-kinase with wortmannin or LY 294002 protein kinase; PKC, protein kinase C; PLC, phospholipase C; MMP, matrix metalloproteinase.

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†‡¶§** The abbreviations used are: COX, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; EP, E prostanoid; PI, phosphatidylinositol; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; AR, amphiregulin; TGF-α, transforming growth factor-α; GPCRs, G-protein-coupled receptors; BSA, bovine serum albumin; PBS, phosphate-buffered saline; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PLC, phospholipase C; MMP, matrix metalloproteinase.
blocked the activation of Akt. These inhibitors also blocked the morphological, proliferative, and metastatic changes induced by PGE₂. From our previous study we found that PGE₂ initially binds to its cognate receptor, EP4 in LS-174T cells, which in turn activates signals that lead to dramatic changes in cell biology. Based on these initial studies, we investigated the pathway through which PGE₂ activates the PI 3-kinase/Akt pathway. We found that the PGE₂-induced activation of Akt and the subsequent increase in migration and invasion occur through a Src/EGFR-mediated event.

EXPERIMENTAL PROCEDURES

Materials—Antibodies to Akt and phospho-Akt were obtained from Cell Signaling (Beverly, MA). Antibodies to EGFR, COX-2, and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to phospho-Tyr and EGFR (neutralizing ab) were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Antibodies to EGF, AR, TGF-α, HB-EGF, betacellulin, and human recombinant TGF-α were obtained from R & D Systems (Minneapolis, MN). PGE₂ was purchased from Cayman Chemical (Ann Arbor, MI), and GM6001 was from Chemicon (Temecula, CA). Genistein, PD 153035, AG 1478, PP2, SQ 22536, and U-73122 were purchased from Calbiochem.

Cell Culture—LS174T cells were purchased from the ATCC (Manassas, VA). LS174T/EGFR (A knock in) and LS174T/EGFR ΔiC-30 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to EGFR, COX-2, and β-actin were obtained from Cell Signaling (Beverly, MA). Antibodies to EGFR, COX-2, and β-actin were obtained from Cell Signaling (Beverly, MA). Antibodies to phospho-Tyr and EGFR (neutralizing ab) were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Antibodies to EGF, AR, TGF-α, HB-EGF, betacellulin, and human recombinant TGF-α were obtained from R & D Systems (Minneapolis, MN). PGE₂ was purchased from Cayman Chemical (Ann Arbor, MI), and GM6001 was from Chemicon (Temecula, CA). Genistein, PD 153035, AG 1478, PP2, SQ 22536, and U-73122 were purchased from Calbiochem.

Cell Culture—LS174T cells were purchased from the ATCC (Manassas, VA). Geneticin-resistant number AK025754 was used as a housekeeping gene for normalization of EGFR family receptor and ligand gene expression. Proto/protomer probes for FLJ22101 (GenBank™ accession number FLJ22101) and FLJ22102 (GenBank™ accession number AK025754) were used as a housekeeping gene for normalization of EGFR family receptor and ligand gene expression. Proto/protomer probes for FLJ22101 (GenBank™ accession number FLJ22101) and FLJ22102 (GenBank™ accession number AK025754) were used as a housekeeping gene. Immunoassays were performed with inhibitors and/or agonists at 37 °C for various times as indicated in the experiments. The cells were rinsed twice in ice-cold PBS and scraped in 500 µl of lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 10 µg/ml leupeptin, 10 µg/ml antipain, 1 mM phenylmethylsulfonyl fluoride, 500 µM sodium orthovanadate, and 10 mM β-glycerophosphate). The cells were disrupted by brief sonication and centrifuged at 10,000 × g for 10 min. The supernatant was incubated overnight with gentle rocking at 4 °C with EGFR antibody conjugated to protein A-agarose beads. The beads were collected by centrifugation and washed 3 times with IP buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 10% glycerol, 0.25% sodium deoxycholate, 10 µg/ml leupeptin, 10 µg/ml antipain, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate). PGE2-Induced Transactivation of EGFR

RESULTS

Activation of Akt by Prostaglandin E₂—Previously our laboratory has shown (19) that the treatment of colorectal carcinoma cells with PGE₂ induced the activation of the PI 3-kinase/Akt signaling pathway. However, the earliest time point measured in our original publication was at 2 h following treatment. In order to analyze the earliest PGE₂-induced signaling events affected, we examined the ability of PGE₂ to activate Akt in a short time course as determined by the phosphorylation of Akt on Ser-473. The phosphorylation of Akt on Ser-473 is required for its activation (21) and commonly used as a marker for increased signaling via this pathway. As shown in Fig. 1A, PGE₂ induced the phosphorylation/activation of Akt as early as 1 min after stimulation. The activation of Akt was maximal within 10 min and was sustained through 60 min of treatment. Furthermore, the addition of PGE₂ activated Akt in a dose-dependent manner (Fig. 1B). The activation of Akt was maximal at 1 µM PGE₂ so this concentration of PGE₂ was used in all subsequent experiments. Cell Migration and Invasion Assays—Cell migration and invasion assays were performed as previously described (19). Briefly, 5 × 10⁴ cells were suspended in 400 µl of serum-free McCoy’s 5A medium and placed in the collagen-coated (migration) or 1:10 diluted Matrigel® (invasion) upper chamber. The lower chamber was filled with serum-free McCoy’s 5A medium containing 1 µg PGE₂. After overnight incubation, the cells were fixed in Diff Quick Fixative from Dade Diagnostics (Miami, FL) and stained in 1% crystal violet. Cells were removed from the upper surface of the filter with a cotton swab. Cells were counted on the underside of the filter to determine migration. Values are expressed as the mean ± S.E. of three independent experiments.


A. Tucker, personal communication.
early signaling events mediated by growth factors require tyrosine phosphorylation, we first determined the effect of a general tyrosine kinase inhibitor (genistein) on PGE2-induced activation of Akt. As shown in Fig. 2A, preincubation of LS-174T cells with 10 μM genistein caused a complete inhibition of the PGE2-induced activation of Akt. This indicates an essential role for a tyrosine kinase in this pathway connecting the GPCR (EP4) to PI 3-kinase.

Over the last few years an increasing amount of evidence suggests that GPCRs can transactivate the EGFR (24, 25). To determine whether a functional EGFR was required for the PGE2-induced activation of Akt, we pretreated colonic carcinoma cells with two EGFR kinase-selective inhibitors (PD 153035 and AG 1478). Pretreatment with 1 μM PD 153035 greatly inhibited the activation of Akt by PGE2 (Fig. 2B), although pretreatment with 5 μM AG 1478 completely inhibited the activation of Akt. These results strongly indicate that the PGE2-induced activation of Akt is mediated via the activation of EGFR.

Because the use of EGFR-specific inhibitors blocked the activation of Akt, we next examined whether PGE2 could activate the EGFR directly. Activation of the EGFR was determined by the immunoprecipitation of the EGFR followed by Western analysis with phosphotyrosine-specific antibodies. The treatment of LS-174T cells with 1 μM PGE2-induced a rapid phosphorylation of the EGFR (Fig. 2C). This phosphorylation was detected within 2.5 min and was sustained through 10 min.

Role of Src in the PGE2-induced Transactivation of the EGFR—The activation of Src has been reported to play a role in the GPCR-mediated transactivation of the EGFR (26, 27). Therefore, we determined if the activation of Src is required for the PGE2-induced activation of Akt. As shown in Fig. 3A, pretreatment with the Src-specific inhibitor PP2 completely blocked the activation of Akt by PGE2. Similar results were observed with another inhibitor of Src as well, PP1 (data not shown).

Src has been shown to serve as a signaling mediator between GPCRs and the EGFR as well as a downstream effector of the EGFR. To determine whether the activation of Src in the PGE2-induced activation of Akt lies upstream or downstream of the EGFR, we examined the effect of PP2 on the PGE2-induced phosphorylation of the EGFR. Pretreatment of LS-174T cells with the Src inhibitor PP2 completely inhibited the PGE2-induced phosphorylation of the EGFR (Fig. 3B). As expected, pretreatment with the EGFR-specific inhibitor PD 153035 completely blocked the phosphorylation of the EGFR as well. To verify further the role of Src in the PGE2-induced activation of the EGFR and Akt, we next examined the ability of PGE2 to activate Src by measuring the phosphorylation of Src. As Fig. 3C shows, PGE2 stimulated the phosphorylation of Src in LS-174T cells. Also, pretreatment of the cells with the EGFR-specific inhibitor PD 153035 had little effect on the PGE2-induced phosphorylation of Src. These results indicate that PGE2 transactivates the EGFR through an Src-dependent manner and that Src is activated prior to the EGFR.

PGE2 Transactivates the EGFR through an Intracellular Mechanism—Many possible GPCR signaling pathways have been reported that can transactivate the EGFR. These include the extracellular release of an EGF-like ligand, the activation of a β-arrestin/Src pathway, and the modulation of reactive oxide species that can affect the activity of EGFR-specific ty-
ligands except heregulin, we pretreated cells for 2 h in the presence of individual neutralizing antibodies for the remaining ErbB ligands. Cells were then incubated with 1 μM PGE₂ for 10 min, and the phosphorylation of Akt was determined. As indicated in Fig. 4B, the pretreatment of cells with neutralizing antibodies to AR, TGF-α, HB-EGF, EGF, or betacellulin had no effect on the activation of Akt. The only neutralizing antibody that exhibited any inhibitory effects was the EGFR-neutralizing antibody. This was expected because these antibodies can prevent the dimerization of EGFRs and therefore block receptor activation. Furthermore, PGE₂ has been shown to induce the extracellular release of TGF-α in other colorectal carcinoma cell lines (28). Because we found no effect of TGF-α-neutralizing antibodies in LS-174T cells, we verified the neutralizing ability of the TGF-α antibodies in our system. As shown in Fig. 4C, the addition of TGF-α (1 or 10 μg/ml) induced the phosphorylation of Akt. However, incubation of TGF-α with neutralizing antibodies for 20 min prior to treatment inhibited the activation of Akt completely. Together, these results suggest that the transactivation of the EGFR does not occur to a major extent through the extracellular release of an EGF-like ligand in LS-174T cells.

Although we found no evidence for an extracellular pathway, we could not rule out the possibility that signaling could occur through epiregulin or an unknown ErbB ligand. To explore this possibility further, we also determined what effect a general matrix metalloproteinase (MMP) inhibitor might have on the PGE₂-induced transactivation of the EGFR. The release of EGF and other ErbB ligands has been shown to require the activation of a MMP, like those of the ADAM family (32). As Fig. 4D shows, the pretreatment of LS-174T cells with a general MMP inhibitor, GM6001, had no effect on the PGE₂-induced activation of Akt. This MMP inhibitor also had no effect on the PGE₂-induced phosphorylation of the EGFR (data not shown).

Role of EGFR Transactivation in the PGE₂-induced Migration and Invasion of LS-174T Cells—As discussed previously, we have shown that PGE₂ can induce the migration (uncoated) and invasion (Matrigel-coated) of LS-174T cells through transwell filters (19). To determine whether transactivation of the EGFR is required for the PGE₂-induced migration and invasion of LS-174T cells, we incubated cells in the presence of various inhibitors and determined their effect on cell migration or invasion. As shown in Fig. 5, the EGFR inhibitors (PD 153035 and AG 1478) or Src inhibitors (PP2) can completely block PGE₂-induced migration (Fig. 5A) and invasion (Fig. 5B) of LS-174T cells. However, inhibition of MMPs with GM6001 had no significant effect on migration but did inhibit cell invasion. Because the stimulation of GPCRs can induce the activation of PLC/PKC and cAMP/PKA pathways, we also evaluated the effect of inhibitors to phospholipase C (U-73122) and adenylyl cyclase (SQ 22536). These inhibitors had no effect indicating that these pathways were not involved in PGE₂-stimulated migration or invasion. We have also shown previously that PKA inhibitors did not block the PGE₂-mediated activation of Akt.

Evaluation of EGFR and COX-2 in Human Colorectal Tissues—Our laboratory was the first to report an increase in the expression of COX-2 in colorectal cancers (33). Because COX-2-derived prostaglandins play a role in the migration and invasion of colonic carcinoma cells, and this phenomenon requires the activation of the EGFR, we sought to determine whether there was a correlation between the expression of COX-2 and the EGFR in vivo. As Fig. 6A shows in 3 of 4 samples, we found an increase in the expression of COX-2 that correlated to an increase in the expression of the EGFR in human colorectal cancers. Furthermore, immunoprecipitation
of the EGFR and subsequent phosphotyrosine Western analysis indicated an increase in the activity of the EGFR in 3 of 4 tumor samples versus their adjacent normal mucosa (Fig. 6B). Interestingly, this increase in EGFR activity also correlated with an increase in the expression of COX-2. These results indicate that in vivo COX-2-derived prostaglandins may potentially transactivate the EGFR, and this interaction could affect the biology of carcinoma cells in vivo by increasing their metastatic potential.

**DISCUSSION**

The GPCR-induced transactivation of growth factor receptors is evident in many systems including the H9252-adrenergic, lysophosphatidic acid (Edg), and endothelin receptors (26, 34, 35). Because PGE2 can induce the activation of the PI 3-kinase/Akt pathway and in many circumstances the modulation of Akt signaling normally occurs via growth factor stimulation, we sought to determine whether the effects of PGE2 on LS174T cells were mediated through a growth factor receptor. Our findings are summarized in Fig. 7. Here we report that PGE2 induced the activation of Akt within minutes, and this activation occurred through the EGFR. We show that EGFR-specific

**Fig. 4. Role of ErbB ligands in the PGE2-mediated activation of the Akt signaling pathway.** A, LS-174T cells were treated for 6 h with 1 μM PGE2 or Me2SO (control). mRNA was isolated and used as a template for a real time quantitative reverse-transcription PCR assay to measure the relative levels of the ErbB receptor subtypes and known ErbB ligands. B, LS-174T cells were serum-starved for 48 h and pre-treated with neutralizing antibodies to ErbB ligands (20 μg/ml EGFR, 50 μg/ml AR, 10 μg/ml TGF-α, 20 μg/ml HB-EGF, 50 μg/ml EGF, 10 μg/ml betacellulin (BTC)) prior to stimulation with 1 μM PGE2 or Me2SO (control) for 10 min. Total cellular protein was collected, and equal amounts of protein was separated by SDS-PAGE. Phosphorylated Akt was visualized with anti-phospho-Akt antibodies. The total amounts of Akt in each sample are determined by Western analysis with Akt antibodies. C, LS-174T cells were serum-starved for 48 h and treated for 5 min with either TGF-α (1 or 10 μg/ml) or TGF-α preincubated with TGF-α-specific neutralizing antibodies (10 μg/ml, 20 min). Total cellular protein was collected, and equal amounts of protein were separated by SDS-PAGE. Phosphorylated Akt was visualized with anti-phospho-Akt antibodies. The total amounts of Akt in each sample are determined by Western analysis with Akt antibodies.

**Fig. 5. Role of EGFR and Src in cell migration and invasion.** 5 × 10^4 LS-174T cells were seeded into the upper chamber of modified Boyden chambers in serum-free media (A) or serum-free media with a 1:10 dilution of Matrigel (B). The assay was carried out for 24 h with the presence of 1 μM PGE2 with or without the indicated inhibitors (1 μM PD 153035, 5 μM AG 1478, 10 μM PP2, 10 μM GM6001, 10 μM SQ 22536, or 1 μM U-73122). Migrated cells were counted and averaged from 3 wells from 2 independent experiments.

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amounts of Akt in each sample are determined by Western analysis with Akt antibodies. C, LS-174T cells were serum-starved for 48 h and treated for 5 min with either TGF-α (1 or 10 μg/ml) or TGF-α preincubated with TGF-α-specific neutralizing antibodies (10 μg/ml, 20 min). Total cellular protein was collected, and equal amounts of protein were separated by SDS-PAGE. Phosphorylated Akt was visualized with anti-phospho-Akt antibodies. The total amounts of Akt in each sample are determined by Western analysis with Akt antibodies.
tyrosine kinase inhibitors completely block PGE\(_2\)-induced activation of Akt. Furthermore, PGE\(_2\) itself can induce the phosphorylation and, therefore, activation of the EGFR. Recently, Pai et al. (28) reported a similar finding, consistent with our results, indicating that PGE\(_2\) can transactivate the EGFR. We extended our findings to reveal that PGE\(_2\) transactivates the EGFR via an intracellular Src-mediated pathway.

The ability of GPCRs to transactivate the EGFR can occur through many distinct pathways (reviewed in Refs. 22 and 36). One example is the extracellular release of EGF and other ErbB ligands through MMPs such as ADAM. The activation of MMPs by GPCRs occurs via PKC, Src, Pyk-2, or reactive oxide species. Furthermore, the transactivation of the EGFR can also occur through intracellular pathways including \(\beta\)-arrestin/Src, \(\beta\gamma\) subunit/Src, and the inhibitory effects of reactive oxide species on EGFR-specific phosphatases. We report here that the PGE\(_2\)-induced transactivation of the EGFR in LS-174T cells occurs through the activation of Src. The use of Src-specific inhibitors can block the activation of Akt by PGE\(_2\). Also, PGE\(_2\) can induce the phosphorylation of Src on Tyr-418 which is a marker for Src activation. Because Src can act as a downstream effector of the EGFR, we sought to determine precisely where Src exerts its effects in the signaling cascade. We report here that the inhibition of Src completely blocks the activation of the EGFR. Furthermore, the inhibition of the EGFR had little effect on the ability of PGE\(_2\) to induce the activation of Src. Together, these data demonstrate that Src is a key mediator between the EP receptor and the EGFR. Although the direct mechanism for the EP receptor-mediated activation of Src was not determined in this study, it is one aspect of our ongoing investigation to understand the role of PGE\(_2\) in colorectal carcinogenesis.

As indicated earlier, Src has been shown to be involved in both the activation of MMPs and subsequent release of ErbB ligands as well as the direct phosphorylation and activation of the EGFR. In order to examine the exact role of Src in this system, we determined the effect of neutralizing antibodies to EGF and EGF-like ligands in the PGE\(_2\)-induced activation of Akt. The addition of neutralizing antibodies that bind specifically to individual members of the EGF family of ligands will block their association with the EGFR and inhibit its activation. We found that the addition of neutralizing antibodies to EGF, TGF-\(\alpha\), amphiregulin, HB-EGF, or betacellulin had no effect on the ability of PGE\(_2\) to induce the activation of Akt, although LS-174T cells produce mRNAs for all ligands. Even though the EP receptor has been shown by others to transactivate the EGFR through the extracellular release of TGF-\(\alpha\) (28), none of the neutralizing antibodies we used had any effect on the PGE\(_2\)-mediated activation of Akt (Fig. 4B). To confirm our results, we verified the ability of TGF-\(\alpha\) neutralizing antibodies to inhibit TGF-\(\alpha\) signaling in our system (Fig. 4C). In addition, pretreatment of LS-174T cells with neutralizing antibodies to TGF-\(\alpha\) had no effect on the ability of PGE\(_2\) to induce migration (data not shown). The disparity in this finding is most likely due to cell type-specific differences and serves to underline the diversity of signaling events within colorectal carcinomas.

Furthermore, we could not evaluate the effect of neutralizing antibodies to epiregulin because none are currently available. This would have been an interesting experiment because the LS-174T cells were shown to express high levels of epiregulin mRNA. Due to the inability of the MMP inhibitor, GM6001, to block the activation of Akt, we believe that the signaling events of PGE\(_2\), at least in these cells, occur prominently through an intracellular pathway.

Our data also demonstrate the involvement of Src and the EGFR in the PGE\(_2\)-induced migration and invasion of colorectal carcinoma cells. We found that the addition of the EGFR-specific tyrosine kinase inhibitors PD 153035 and AG 1478 completely inhibited the PGE\(_2\)-induced migration and invasion of LS-174T cells. Similarly, we also found that the Src inhibitor, PP2, completely inhibited cell migration and invasion. We found similar effects with another Src inhibitor, PP1 (data not shown). The ability of the EGFR and Src inhibitors to completely block cell migration and invasion indicates that these mediators lie within the same signaling cascade. Conversely, the addition of the general MMP inhibitor GM6001 had no significant effect on cell migration although greatly inhibiting cell invasion. This indicates that MMP activity is not required for transactivation of the EGFR and cell migration but is required for cell invasion. This was expected because the activation of MMP-9 has been shown to be required for cell migration
been shown, such as the activation of Src through that can mediate the GPCR to EGFR signaling cascade have the EGFR can reduce the polyp formation in APCMin/ was found to be highly phosphorylated. Hence, important in tumor samples. Furthermore, in the same tissues the EGFR human colorectal specimens and found a correlation between expression of COX-2 in normal and tumor sections from 4 other reported pathways that can target both the COX-2 and EGFR pathways. H-89, or the PKC inhibitor, Ro-31-8220, to block the activation of Akt by PGE2 (data not shown). Other reported pathways currently under investigation in our laboratory.

Since the first discovery of an increase in COX-2 expression in human cancers, the exact role of COX-2 and its derived prostaglandins have been the focus of many studies. To investigate if the transactivation of the EGFR by PGE2 might serve a functional role in colorectal carcinoma formation in vivo, we analyzed the expression and activation of EGFR versus the expression of COX-2 in normal and tumor sections from 4 human colorectal specimens and found a correlation between increased expression of COX-2 and the EGFR in 75% of the tumor samples. Furthermore, in the same tissues the EGFR was found to be highly phosphorylated. Hence, important interactions between these pathways may occur in vivo as well.

Recently, Torrance et al. (38) have shown that the use of a non-selective COX inhibitor in combination with an inhibitor to the EGFR can reduce the polypl formation in APCMin-/ mice much more effectively than either agent alone. In light of these studies, our findings that the PGE2-induced transactivation of the EGFR is required for stimulation of LS-174T cell migration and invasion are quite intriguing. In conclusion, our data demonstrate a direct intracellular communication further supporting the combinational use of COX-2 and EGFR inhibitors for cancer prevention or treatment. We strongly support clinical studies that would further evaluate the combination of agents that target both the COX-2 and EGFR pathways.

REFERENCES