Prostaglandin E₂ Promotes Lung Cancer Cell Migration via EP4-βArrestin1-c-Src Signalsome

Jae Il Kim, Vijayabaskar Lakshmikanthan, Nicole Frilot, and Yehia Daaka

Abstract

Many human cancers express elevated levels of cyclooxygenase-2 (COX-2), an enzyme responsible for the biosynthesis of prostaglandins. Available clinical data establish the protective effect of COX-2 inhibition on human cancer progression. However, despite these encouraging outcomes, the appearance of unwanted side effects remains a major hurdle for the general application of COX-2 inhibitors as effective cancer drugs. Hence, a better understanding of the molecular signals downstream of COX-2 is needed for the elucidation of drug targets that may improve cancer therapy. Here, we show that the COX-2 product prostaglandin E₂ (PGE₂) acts on cognate receptor EP4 to promote the migration of A549 lung cancer cells. Treatment with PGE₂ enhances tyrosine kinase c-Src activation, and blockade of c-Src activity represses the PGE₂-mediated lung cancer cell migration. PGE₂ affects target cells by activating four receptors named EP1 to EP4. Use of EP subtype-selective ligand agonists suggested that EP4 mediates prostaglandin-induced A549 lung cancer cell migration, and this conclusion was confirmed using a short hairpin RNA approach to specifically knock down EP4 expression. Proximal EP4 effectors include heterotrimeric Gs and βArrestin proteins. Knockdown of βArrestin1 expression with shRNA significantly impaired the PGE₂-induced c-Src activation and cell migration. Together, these results support the idea that increased expression of the COX-2 product PGE₂ in the lung tumor microenvironment may initiate a mitogenic signaling cascade composed of EP4, βArrestin1, and c-Src which mediates cancer cell migration. Selective targeting of EP4 with a ligand antagonist may provide an efficient approach to better manage patients with advanced lung cancer. Mol Cancer Res; 8(4); 569–77. ©2010 AACR.

Introduction

Cancer diseases claim over half a million lives in the United States annually, and lung cancer is the number one cause of death in both men and women. Limited success in the effectiveness of lung cancer treatment is due in part to the cancer cells' ability to spread and metastasize very early in the disease course. Accumulating epidemiologic and clinical data provide a strong link between inflammation and cancer initiation or progression, but the molecular inflammatory determinants remain to be established. Nonetheless, the importance of the tumor microenvironment and inflammation in neoplastic progression is evident from studies of cancer risk among nonsteroidal anti-inflammatory drug users, who experience reduced risk for many types of cancers (1).

One of the primary mechanisms underlying the chemopreventive effects of nonsteroidal anti-inflammatory drugs lies in their ability to inhibit cyclooxygenase enzymes COX-1 and COX-2 activation. Whereas COX-1 is expressed constitutively, the COX-2 protein is usually not detected in normal tissues but is instead inducible by cytokines and growth factors at sites of inflammation (2-6). Indeed, COX-2 protein levels are elevated in several cancer types, including colorectal, prostate, and lung cancers (7, 8), and suppression of either COX-2 expression or COX-2 activation, may be effective in cancer prevention and therapy as it promotes the repression of a variety of cancer hallmark traits such as angiogenesis and metastasis (4, 9, 10). Alas, in spite of hopeful results, the long-term use of selective COX-2 inhibitors as an effective therapeutic approach to manage cancer progression has been questioned due to unwanted side effects such as increased cardiovascular risks (8-13).

COX-2 uses plasma membrane–expressed arachidonic acid as a substrate to generate lipid mediators that are rapidly converted to prostaglandins (PG), i.e., PGD₂, PGE₂, PGF₂, PGI₂, and TXA₂. The prostaglandins exert important biological effects in target organs, such as in the regulation of immune function, gastrointestinal homeostasis,
and inflammation (4). It is hypothesized that the cardiovascular risks associated with COX-2–selective inhibition may result, at least in part, from an imbalance created between PGI2 and TxA2, both of which possess key physiologic roles in vasoregulation and platelet aggregation (1, 8, 12). Hence, a better understanding of COX-2 signaling, and identification of its downstream effector(s), is essential for developing effective drugs that aim to circumvent the risk of unwanted cardiovascular events associated with the selective inhibition of COX-2.

Of the five prostaglandins produced by COX-2, PGE2 is the predominant one associated with cancer (14-20). Four receptor subtypes that belong to the seven transmembrane–spanning G protein–coupled receptor (GPCR) superfamily are known to bind PGE2, and they are named EP1 to EP4. Upon binding PGE2, each EP subtype transduces signals through distinct heterotrimeric G proteins: EP1 signals through Gq, EP2 and EP4 signal through Gs, and EP3 signals through Gi. In addition, stimulation with PGE2 may potentiate Wnt signaling or transactivate other types of receptors, including receptor tyrosine kinases (1), albeit by mechanisms not fully understood. In this study, we examined the role of PGE2 in lung cancer cell migration. In A549 lung cancer alveolar cells, COX-1 is constitutively expressed whereas COX-2 expression is inducible (5). The A549 cells secrete little PGE2, and instead, it seems that COX-2 induction is largely responsible for PGE2 production (5, 6). Using A549 cells as a model, we found that these cells express all four EP subtypes but that only selective activation of EP4 could lead to increased cell migration. Furthermore, we found that stimulation with PGE2 could activate the non–receptor tyrosine kinase c-Src which is required for PGE2–induced A549 cell migration. Remarkably, PGE2–mediated c-Src activation and A549 cell migration failed when the expression of the adaptor protein β-Arrestin1 was knocked down with short hairpin RNA (shRNA). These results provide a rationale for considering the EP4–β-Arrestin1 signalosome as an effective drug target to more effectively treat patients with advanced lung cancer.

Materials and Methods

Cell Culture, Antibodies, and Reagents

A549 and HEK293T cells were obtained from the American Type Culture Collection. A549 cells were maintained in F12K medium containing 10% fetal bovine serum, 100 units/mL of penicillin, and 100 μg/mL of streptomycin at 37°C with 5% CO2. F12K medium supplemented with 0.1% bovine serum albumin (BSA), 100 units/mL of penicillin, and 100 μg/mL of streptomycin was used as starvation medium for the A549 cells. HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL of penicillin, and 100 μg/mL of streptomycin at 37°C with 5% CO2. All cells were maintained at 60% to 80% confluency, except for cells in the wound-healing assay (95-100% before wounding). PGE2, butaprost, sulprostone, and PGE1-OH were purchased from Cayman Chemicals; BSA, polybrene, and puromycin were from Sigma Aldrich; TRizol and epidermal growth factor were from Invitrogen; FuGENE HD transfection reagent was from Roche Applied Science; F12K and DMEM were from Cellgro; and fetal bovine serum was from HyClone. Antibodies used were anti-c-Src sc-18, anti-ERK2 sc-154 (Santa Cruz); anti-p-Tyr 4G10 (Millipore); anti-GAPDH, anti-β-actin, and anti-p44/42 (Cell Signaling); anti–EP1-4 (Cayman Chemicals); and anti-FAK (BD Biosciences).

Cell Proliferation

Cell proliferation was determined by counting cells that excluded trypan blue (Invitrogen) with a hemacytometer, and treated under the same conditions described for wound healing assay (described herein). Briefly, cells were trypsinized and resuspended in growth medium, aliquots prepared in a 1:1 ratio of 2× trypan blue, and cells counted using a hemacytometer. An equal number of cells (5×10⁴ cells/well) was resuspended in medium containing the indicated agents and seeded in six-well plates. These experiments were done thrice in duplicate for analysis.

Immunoblotting and Immunoprecipitation Assays

Cells grown on 100 mm dishes were washed with PBS and harvested in lysis buffer containing 25 mmol/L of Tris (pH 8.0), 100 mmol/L of NaCl, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 1 mmol/L of EDTA, 1 mmol/L of phenylmethylsulfonyl fluoride, 10 μg/mL of aprotinin, 10 μg/mL of leupeptin, and 2 μg/mL of pepstatin A. For detection of phosphorylated proteins, phosphatase inhibitor mixture (P1 and P2, 1:1,000; Sigma) and 1 mmol/L of sodium orthovanadate were included in the lysis buffer. For immunoprecipitation, the cell lysates were cleared by centrifugation at 10,000 × g for 15 min at 4°C, incubated with anti–c-Src antibody overnight, and the proteins were precipitated and eluted with 70 μL of a 30% slurry of protein G plus/protein A-agarose beads (Calbiochem) and rotated for 4 h at 4°C. Immune complexes were washed thrice with ice-cold glycerol lysis buffer containing 0.1% (w/v) SDS, and the samples were then denatured in Laemmli sample buffer by boiling for 5 min. Before immunoprecipitation, 50 μL of the cell lysate were aliquoted to provide samples for c-Src expression determined by immunoblotting. Proteins (immunoprecipitated with c-Src antibody as well as from total cell lysates) were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad), and immunoblotted with 1:1,000 dilutions of primary antibodies for the protein of interest. Horseradish peroxidase–conjugated anti-rabbit/mouse secondary antibodies (Jackson ImmunoResearch) were diluted 1:10,000 in blocking buffer (3% BSA-TBST) and blots incubated for 1 h at room temperature. All antibodies were diluted in 3% BSA-TBST. Immunoblots were visualized using Amersham ECL plus chemiluminescence reagent (GE Healthcare).
**Wound Healing Assay**

A549 cells were seeded in six-well plates and incubated overnight in starvation medium. Cell monolayers were wounded with a sterile 200-μL pipette tip, washed with starvation medium to remove detached cells, and stimulated with the indicated agonist for 24 h. Where indicated, antagonists were added to the cells 30 min before stimulation. Next, cells were fixed in 4% formalin, stained with hematoxylin, counterstained with bluing reagent (Richard-Allan Scientific), washed with water, and air dried. Cells were photographed using a digital camera (Nikon) integrated in a bright-field microscope (Zeiss).

**Cell Migration**

Cell migration assays were done using 8-μm-pore transwell chambers (Costar). Briefly, A549 cell monolayers were detached using HyQtase (Hyclone), resuspended in starvation medium, and cells added to the transwell chambers (2 × 10^5 cells/well). Starvation medium containing the indicated agonists was added to the lower chambers, and transwells placed in contact with the agonists at 37°C for 5 h. Where indicated, the antagonists were added to cells 30 min before stimulation. Cells that migrated to the lower surface of the filter were fixed with 3.7% paraformaldehyde, stained with 0.5% crystal violet, and migrated cells counted through a 400× objective with an Axioskop microscope (Zeiss).

**Knockdown shRNA**

For stable knockdown by shRNA, we screened five lentiviral DNA expression vector sets that each contained 21 nucleotide shRNA duplexes that targeted our genes of interest (Open Biosystems). The shRNA vectors were co-transfected with equal concentrations of helper plasmids VSVG and delta 8.9 into HEK293T cells for lentivirus production using FuGENE HD. As a negative control, HEK293T cells were transfected with the same amount of expression vector containing the green fluorescent protein targeting sequence, 5'-GCA AGC TGA CCC TGA AGT TCA T-3'. Virus-containing medium from transfected HEK293T cells were collected 24 h after transfection and mixed with 5.0 mg of polybrene for infection of A549 cells. HEK293T cells were replenished with fresh medium for another 24 h, and the virus-containing medium was collected for a second infection with polybrene. Cells were infected for 2 h, and grown in F12K medium containing 10% fetal bovine serum for 48 h prior to selection with puromycin (5 μg/mL). Uninfected A549 cells were used as a selection control. Cells were maintained in puromycin (1 μg/mL), and protein expression of target genes was determined by Western blotting. The cells with optimal knockdown (>50% reduction) were used for experiments (Open Biosystems: ARRB1 shRNA clone TRCN0000000161, PTGER4 shRNA clone TRCN0000000204).

**PCR Amplification**

Total RNA was isolated with TRIzol Reagent, as described by the manufacturer (Invitrogen). The RNA was
reverse transcribed (Invitrogen RT-Superscript III Kit) to prepare the cDNA to be amplified by PCR, using specific sense and antisense primers for the \textit{ARRB1}, \textit{ARRB2}, and housekeeping gene \textit{GAPDH}. Primers were synthesized by Integrated DNA Technologies as follows: $\beta$Arrestin1 exon 8 (forward GGT AAT AGA TCT CCT TAT CC; reverse CCA CAA GCG GAA TTC TGT G), $\beta$Arrestin2 exon 9 (forward GTA CTG TCT CAC AGA GAC TTT; reverse GAC AAG GAG CTG TAC TAC CA), and GAPDH (forward CAT GGG TGT GAA CCA TGA GAA; reverse GGT CAT GAG TCC TTC CAC GAT).

Statistics and Quantifications

Data are presented as the mean ± SE of three independent experiments. The data was analyzed for one-way ANOVA and followed by the Tukey post-test to determine the statistical significance. All statistical analyses were done, and all graphs generated, using GraphPad Prism 5.0 software (GraphPad). The x and y labels of all presented data were prepared using Adobe Illustrator CS2 (Adobe).

Results

PGE$_2$ Increases Migration of Lung Adenocarcinoma A549 Cells

PGE$_2$ regulates cancer cell proliferation and survival by multiple mechanisms, and we asked if it also affects cell migration. Exposure to PGE$_2$ enhanced the migration of alveolar cell carcinoma A549 cells in a dose-dependent manner detectable by both wound healing (Fig. 1A) and

![Figure 2](image-url)
transwell migration (Fig. 1B) assays. Because A549 cells are capable of migrating under basal conditions and PGE₂ can increase the cancer cell proliferation (21-23), we tested whether this lipid mediator could actually be increasing the number of basal migratory cells. Under the conditions used in our study, stimulation with PGE₂ did not seem to significantly affect the rate of A549 cell proliferation in comparison with vehicle-treated cells (Fig. 1C). We therefore conclude that PGE₂ specifically augments the inherent migration character of lung cancer A549 cells.

EP₄ Mediates the Cell Migration

PGE₂ binds to and activates four distinct receptor subtypes named EP1 to EP4. Upon activation by PGE₂, EP2 and EP4 couple to heterotrimeric Gs proteins that activate adenylate cyclase to synthesize cyclic AMP. EP1 and EP3, on the other hand, transmit signals via Gq and Gi proteins to transiently increase intracellular Ca²⁺ levels or inhibit adenylate cyclase, respectively (16, 24, 25). To begin to elucidate the mechanisms involved in PGE₂-mediated lung cancer cell migration, we asked which EP subtype(s) are expressed in the A549 cells and are responsible for mediating the cell migration. Using Western blot analysis, we were able to show the expression of all four EP subtypes (Fig. 2A). Next, we used selective EP subtype ligand agonists and show that A549 cells stimulated with EP1/EP3-selective sulprostone or EP2-selective butaprost failed to migrate (Fig. 2B and C). Distinctly, the selective activation of EP4 with PGE₁-OH significantly enhanced the migration of A549 cells as measured by wound healing (Fig. 2B) or transwell (Fig. 2C) migration assays.

Because PGE₂-mediated activation of EP4 signaling does not necessarily exclude activation of the other EPs, we confirmed the selective role of EP4 in the PGE₂-induced cell migration by using shRNA. A549 cells stably expressing shRNA targeting the PTGER4 gene yielded ~60% reduction in the EP4 protein expression (Fig. 2D, inset), and these cells failed to migrate following stimulation with either PGE₂ or PGE₁-OH (Fig. 2D). The EP4 knockdown did not affect basal cell migration, suggesting that EP4 only regulates ligand agonist-mediated A549 cell migration. Because PGE₂ treatment of EP4 knockdown cells does not exclude the availability of EP1 to EP3 for activation but failed to enhance cell migration, we conclude that selective activation of EP4 is central to PGE₂-provoked migration of the A549 cells.

PGE₂-Induced Cell Migration is Independent of Extracellular Signal-Regulated Kinase Activation

Growth factors and cytokines transmit oncogenic signals by activating the Ras-extracellular signal regulated kinase 1 and 2 (ERK) signalsome (26). The ERK may be activated in response to GPCR signaling, and ERK activity is required for the migration of many cancer cell types (26-29). In the case of A549 lung cancer cells, PGE₂ acts primarily on EP4 to enhance cell migration, and we tested whether treatment with PGE₂ could affect the ERK activation state. The results show that treatment with either PGE₂ (data not shown) or PGE₁-OH (Fig. 3A) leads to

![Figure 3](https://www.aacrjournals.org/mcr/article-figs/figure3.png)

**FIGURE 3.** ERK activation is not required for the prostaglandin-enhanced cell migration. A, PGE₁-OH promotes time-dependent phosphorylation of ERK in A549 cells. Cells were incubated in starvation medium overnight and treated with PGE₁-OH (1 μmol/L) for the indicated times. Cell monolayers were washed once with ice-cold PBS, lysed with radioimmunoprecipitation assay buffer, and analyzed by Western blotting using anti-phosphorylated ERK antibodies. The same filter was also analyzed for the expression of total ERK2 to control for equal protein loading in each lane. B, PD98059 inhibits the PGE₂-mediated ERK phosphorylation. Cells were incubated in starvation medium overnight and resuspended in medium containing either vehicle or the indicated concentrations of PD98059 for 30 min prior to treatment, or not, with PGE₂ (1 μmol/L) for 10 min. ERK phosphorylation was determined as described above. C, activated ERK does not affect PGE₂-mediated lung cancer cell migration. A549 cells were pretreated with PD98059 (50 μmol/L) for 30 min and analyzed for cell migration using the transwell assay. These experiments were repeated thrice in duplicate and the data are presented as fold increase over vehicle-treated control. *, P < 0.05 compared with vehicle control.
ERK phosphorylation in a biphasic manner reminiscent of similar activation patterns seen with ligand-induced stimulation of other GPCRs (30). The existence of both rapid and sustained ERK activation has been explained in previous studies to be mediated by G protein–dependent or βArrestin–dependent pathways, respectively (27, 28). Based on this knowledge, we conclude that stimulation with PGE2 promotes signaling through both G proteins and βArrestin.

To determine if PGE2-induced ERK activation is responsible for the A549 cell migration, we used PD98059, a small molecule MEK inhibitor, to suppress ERK phosphorylation. Pretreatment with the PD98059 for 30 minutes inhibited the PGE2-induced ERK phosphorylation (Fig. 3B) and basal level of cell migration (Fig. 3C), but failed to affect the PGE2-mediated cell migration (Fig. 3C). PD98059 (10, 25, or 50 μmol/L) exerted no cytotoxic effect on A549 cells (data not shown). Together, these results indicate that the activation of ERK by PGE2 stimulation does not play a significant role in the A549 lung cancer cell migration.

PGE2 Induces Cell Migration via βArrestin1 and Src Activation

The cellular Src (c-Src) tyrosine kinase has well-established roles in the progression of many different human cancers (31–34). In particular, an increase in c-Src protein levels and tyrosine kinase activity has been shown to promote tumor cell metastasis, whereas inhibition of c-Src activation leads to decreased tumor cell migration and invasion (35). Our results show that stimulation with PGE2 promotes the latent ERK phosphorylation (Fig. 3A) that is likely mediated through βArrestin-dependent signals (30), and we have previously reported that βArrestin1 directly interacts with c-Src and increases the c-Src tyrosine kinase activity (36). We show here that PGE2 treatment similarly causes an increase in c-Src tyrosine phosphorylation in a time-dependent manner in the A549 lung adenocarcinoma cells (Fig. 4A). Importantly, blockade of c-Src activation by pretreatment with the small molecule c-Src inhibitor PP2 (Fig. 4B) completely abolished the ability of PGE2 to promote A549 cell migration (Fig. 4C and D), but showed no effect on A549 cell viability (data not shown). These results

**FIGURE 4.** PGE2 promotes c-Src–dependent lung cancer cell migration. A, PGE2 promotes c-Src tyrosine phosphorylation. A549 cells were incubated in starvation medium overnight prior to treatment with PGE2 (1 μmol/L) for the indicated times or with epidermal growth factor (EGF; 10 ng/mL) for 5 min. Cell monolayers were washed once with ice-cold PBS and lysed with radioimmunoprecipitation assay buffer. Protein extracts were quantified by the Bradford method, and 1 mg of total protein was used for immunoprecipitation with anti–c-Src antibody. Immunoprecipitates were washed, separated on SDS-PAGE, and analyzed by Western blotting with anti–phosphorylated tyrosine (4G10) antibody (top). Total cell lysates were probed with anti–c-Src antibody (bottom). B, PP2 inhibits the PGE2-mediated c-Src phosphorylation. Cells were incubated in starvation medium overnight and pretreated with PP2 at the indicated concentrations for 30 min prior to treatment with PGE2 (1 μmol/L) for 5 min. Immunoprecipitates were probed for tyrosine phosphorylation of c-Src, as described. C and D, PP2 inhibits the PGE2-mediated cell migration. A549 cells were pretreated with PP2 (10 μmol/L) for 30 min prior to treatment with PGE2 or epidermal growth factor (10 ng/mL) and analyzed for migration using the wound healing (C) and transwell (D) assays. Experiments were repeated thrice in duplicate and data are presented as fold increase above vehicle-treated samples. *, P < 0.05 compared with vehicle control.
show that activation of c-Src by PGE₂ is critical for the migration of lung cancer A549 cells.

Desensitization of GPCR signaling occurs in a stepwise manner that involves phosphorylation of the agonist-bound receptor by GPCR kinases and subsequent binding of βArrestins to the receptor. With βArrestins bound, the GPCRs are physically uncoupled from further interaction with G proteins. In addition to desensitization, the βArrestins could act as scaffolds for a diverse array of signaling pathways and are increasingly becoming appreciated as critical regulators of directed cell chemotaxis and migration (37). Our results show that stimulation with PGE₂ activates c-Src (Fig. 4A), and βArrestin1 interacts with c-Src and activates it (36). Hence, we established if βArrestin1 plays a role in the PGE₂-induced migration of lung cancer A549 cells. First, we documented the expression of the βArrestin1 gene (Fig. 5A) and protein (Fig. 5B) in these cells. Next, we used a lentiviral shRNA delivery system to create a stable A549 cell line with reduced βArrestin1 expression (Fig. 5B). Having shown that c-Src activation is required for PGE₂-induced cell migration (Fig. 4D), and that βArrestin1 potentiates c-Src activation (36), we tested the effect of βArrestin1 knockdown on c-Src activation in A549 cells. Our results show that the knockdown of βArrestin1 expression attenuates the PGE₂-induced tyrosine phosphorylation of c-Src (Fig. 5C) and significantly suppresses cancer cell migration (Fig. 5D). Collectively, these results indicate that in A549 cells, βArrestin1 is essential for the PGE₂-mediated activation of c-Src and increase in cell migration.

Discussion

COX-2 expression is increased in human lung cancer tissues (37) and it is a principal enzyme in the biosynthesis of the prostaglandins, including PGE₂ (36). Under physiologic conditions, PGE₂ activates four cognate receptors that are collectively involved in the regulation of cellular homeostasis. However, uncontrolled signaling by the PGE₂ receptors has been implicated in the initiation and progression of several human diseases including cancer, but the mechanisms involved remain to be uncovered. In this study, evidence is presented to show that PGE₂ activates its cognate EP4 receptor subtype to induce the migration of lung cancer A549 cells by activating c-Src through a mechanism that involves the adaptor protein βArrestin1 (Fig. 6).

The nature and magnitude of receptor-induced signals are directly linked to the production and availability of ligand agonists. The production of prostanooids is initiated by cytosolic phospholipase A2–mediated release of arachidonic acid from phospholipids (38). The liberated arachidonic acid can be oxygenated via the cyclic prostaglandin synthase COX-2 pathway into intermediary lipids that are converted into individual prostaglandins by respective synthases (4). Significantly, several types of human cancers express elevated levels of COX-2 and outcome data show the protective effects of pharmacologic COX-2 inhibitors in preventing tumor induction as well as inhibiting tumor growth, providing a rationale to use COX-2 inhibitors as cancer therapeutics. However, long-term clinical use of selective COX-2 inhibitors has been questioned due to associated health risks (39). In this report, we show that the COX-2 product PGE₂, the expression of which is elevated in human cancers (7, 8, 40), is efficient at inducing lung cancer cell migration. These results suggest that the specific targeting of PGE₂ signals may provide a way to harness the beneficial effects of inhibiting COX-2 activity (i.e., limit tumor induction and progression) but circumvent the COX-2 inhibitor-associated unwanted health risks.

These results provide a rationale for the blockade of EP4 signaling events with specific ligand antagonists as a therapeutic modality to interfere with lung cancer progression.

The classic signal transduction from GPCRs to G proteins is rapidly desensitized in a highly conserved process that involves phosphorylation of agonist-occupied receptors by GPCR kinases. The GPCR kinases phosphorylate residues in the intracellular loop and carboxyl tail of the receptor leading to the recruitment of cytosolic β Arrestins to the receptor. The binding of β Arrestins to GPCR kinase-phosphorylated receptors sterically hinders receptor-induced G protein activation. In addition to inhibiting the receptor-mediated G protein activation, β Arrestins may also mediate G protein-independent signals (26, 27). This can be explained in part by the knowledge that β Arrestins are multifunctional proteins that, in addition to their classic roles as GPCR-desensitizing molecules, could act as scaffolds and adaptor proteins that facilitate a growing number of signaling events. For example, we previously showed that β Arrestin1 mediates the β2 adrenergic receptor–induced activation of c-Src by forming a receptor-anchored signalosome containing β Arrestin1 and c-Src (36). A subsequent study showed that PGE2 uses a similar mechanism to induce colon cancer cell migration, but the identity of the EP subtype responsible has not been elucidated (25). EP4 has been shown to be involved in colon carcinoma cell motility, growth, and anchorage independence (42-44). In this study, we showed that PGE2 promotes the c-Src activation via EP4, providing a rationale for its use as a therapeutic target to treat patients with advanced lung cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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