Prostaglandin E<sub>2</sub> Selectively Antagonizes Prostaglandin F<sub>2α</sub>-stimulated T-cell Factor/β-Catenin Signaling Pathway by the FP<sub>B</sub> Prostanoid Receptor*•

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FP prostanoid receptors are G-protein-coupled receptors that consist of two isoforms named FP<sub>A</sub> and FP<sub>B</sub>. Both isoforms activate inositol phosphate second messenger signaling pathways by their endogenous ligand prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>). Previously, we have shown that both isoforms undergo Rho-mediated cell rounding following treatment with PGF<sub>2α</sub>. Following the removal of PGF<sub>2α</sub>, however, FP<sub>A</sub>-expressing cells return to their original morphology, whereas FP<sub>B</sub>-expressing cells do not. It was also found that PGF<sub>2α</sub>-could activate T-cell factor (Tcf)/β-catenin signaling in cells expressing the FP<sub>B</sub> isoform but not in cells expressing the FP<sub>A</sub> isoform. We now show that prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) can induce cell rounding and stimulate the formation of inositol phosphates to the same extent as PGF<sub>2α</sub> in cells expressing either the FP<sub>A</sub> or FP<sub>B</sub> isoforms. However, PGE<sub>2</sub> has much lower efficacy as compared with PGF<sub>2α</sub> for the activation of Tcf/β-catenin signaling in FP<sub>B</sub>-expressing cells, and the cell rounding is reversible. Interestingly, pretreatment of FP<sub>B</sub>-expressing cells with PGE<sub>2</sub>-attenuated PGF<sub>2α</sub>-stimulated Tcf/β-catenin signaling in a dose-dependent manner while having no effect on PGF<sub>2α</sub>-stimulated inositol phosphates formation. Thus, the ratio of endogenous PGE<sub>2</sub> and PGF<sub>2α</sub> has the potential to selectively regulate one signaling pathway over another. This represents a novel mechanism for the regulation of cell signaling that is distinct from regulation occurring at the level of the receptor and its effector pathways.

The ovine FP<sub>A</sub> and FP<sub>B</sub> prostanoid receptors are G-protein coupled receptors that are generated by alternative mRNA splicing of their carboxyl termini (1). Relative to one another, the FP<sub>B</sub> isoform represents a FP<sub>A</sub> receptor in which the last 46 amino acids of the carboxyl terminus have been removed and replaced with one amino acid. The endogenous ligand of these receptors is considered to be prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) (2); however, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and PGD<sub>2</sub> can bind to these receptor isoforms with affinities that are only 10–30-fold less than PGF<sub>2α</sub> (1, 2).

The FP<sub>A</sub> and FP<sub>B</sub> receptor isoforms are both coupled to Go<sub>q</sub> and following stimulation by PGF<sub>2α</sub> both will activate phosphatidylinositol hydrolysis. PGF<sub>2α</sub> will also activate the small G-protein, Rho, in cells expressing either the FP<sub>A</sub> or FP<sub>B</sub> receptors (3). The activation of Rho leads to cell rounding and the formation of cobblestone-like aggregates of cells. Interestingly, in FP<sub>A</sub>-expressing cells these shape changes show rapid reversal following the removal of PGF<sub>2α</sub>, but in FP<sub>B</sub>-expressing cells the shape changes are persistent and appear to be irreversible following the removal of PGF<sub>2α</sub>. In FP<sub>B</sub>-expressing cells (but not in FP<sub>A</sub>-expressing cells) PGF<sub>2α</sub> can also produce a marked activation of T-cell factor (Tcf)/β-catenin signaling (5). This activation of Tcf/β-catenin signaling requires the activation of Rho but again is unique for the FP<sub>B</sub> isoform even though both isoforms can activate Rho (6).

As a transcription factor, Tcf regulates the expression of a number of genes, including c-myc, cyclin D1, and cyclooxygenase-2 (COX-2) (7). The regulation of COX-2 expression by Tcf is significant given the ability of the FP<sub>B</sub> receptor to activate Tcf/β-catenin signaling, and we have recently shown that PGF<sub>2α</sub> stimulation of the FP<sub>B</sub> receptor leads to a Rho-dependent activation of COX-2 promoter activity (8). One of the hallmarks of colon cancer as well as other cancers is an up-regulation of COX-2 activity and an increased biosynthesis of various prostanoids, most notably PGE<sub>2</sub> and PGF<sub>2α</sub> (7, 9–11). Increased levels of PGE<sub>2</sub> and PGF<sub>2α</sub> would have the potential to further activate their receptors and thereby establish a positive feedback loop. Experimental evidence supporting the development of such a positive feedback loop involving murine EP<sub>2</sub> receptors and the up-regulation of COX-2 expression has been described (12). Furthermore, the potential of FP and EP prostanoid receptors to form positive feedback loops involving the up-regulation of COX-2 has been described (13).

The phylogeny of the prostanoid receptor family suggests that the primordial prostanoid receptor was an EP subtype whose endogenous ligand is PGE<sub>2</sub>. In addition to its high affinity for the EP receptors, PGE<sub>2</sub> has >100 nM affinity for the FP and DP prostanoid receptors. Given that both PGE<sub>2</sub> and PGF<sub>2α</sub> are frequently up-regulated in cancer, we were interested in the possible interactions of these compounds at the level of the activation of the FP<sub>A</sub> and FP<sub>B</sub> receptor isoforms. We were specifically interested in whether both PGE<sub>2</sub> and PGF<sub>2α</sub> had the same efficacy for the activation of inositol phosphates hydrolysis and Tcf/β-catenin signaling and whether they could both induce changes in cellular morphology. Interestingly, both prostanoids had similar efficacy for the activation of inositol phosphates hydrolysis, but PGE<sub>2</sub> had significantly lower efficacy for the activation of Tcf/β-catenin signaling. Furthermore, pretreatment of FP<sub>B</sub>-expressing cells with PGE<sub>2</sub> inhibited subsequent PGF<sub>2α</sub>-stimulated Tcf/β-catenin signaling without changing the PGF<sub>2α</sub>-stimulated accumulation of total inositol phosphates hydrolysis, but PGE<sub>2</sub> had significantly lower efficacy for the activation of Tcf/β-catenin signaling. Furthermore, pretreatment of FP<sub>B</sub>-expressing cells with PGE<sub>2</sub> inhibited subsequent PGF<sub>2α</sub>-stimulated Tcf/β-catenin signaling without changing the PGF<sub>2α</sub>-stimulated accumulation of total inositol phosphates hydrolysis, but PGE<sub>2</sub> had significantly lower efficacy for the activation of Tcf/β-catenin signaling.
phosphates. Both compounds induced changes in cellular morphology, but the effects of PGE₂ on FPB cells were reversible, whereas the effects of PGF₂α were not.

**EXPERIMENTAL PROCEDURES**

**Total Inositol Phosphates Assay**—HEK-293 cells stably expressing the FP_A and FP_B prostanoid receptor isoforms were generated and cultured as described previously (3). These two cell lines have comparable levels of receptor expression judged both by radioligand binding (FP_A, 3.55 ± 0.28 pmol/mg protein; FP_B, 4.09 ± 0.49 pmol/mg protein) and by PGF₂α-stimulated inositol phosphate formation (14). Receptor-stimulated total inositol phosphates accumulation was determined by anion exchange chromatography as described previously (14). Cells were plated and incubated overnight with 3 μCi/ml myo-[32P]inositol. Cells were then trypsinized and centrifuged, and aliquots of 0.5 to 1.0 × 10⁶ cells were resuspended in 500 μl of Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10 mM LiCl. After drug additions, the cells were incubated for 1 h at 37°C, and 2.5 ml of chloroform/methanol/water (1:1:0.5) was added. 900 μl of the aqueous phase was removed and mixed with 2 ml of water and applied to a 2.5-ml column of AG1-X8 anion exchange resin. After three washes with 5 ml of water and two washes with 5 ml of 5 mM borax/60 mM sodium formate buffer, the ³²P-labeled total inositol phosphates were eluted with 2 ml of 0.2 M ammonium formate/0.1 M formic acid, and radioactivity was determined by liquid scintillation counting.

**Cell Imaging**—Cells were plated in six-well tissue culture dishes under half-confluent conditions and were grown without any changes of media for 3–4 days as described previously (4). To examine agonist-induced cell rounding and its reversal, either vehicle (0.002% sodium carbonate or 0.1% Me₂SO, final concentration, respectively) or agonist (1 μM PGF₂α or 1 μM PGE₂, final concentration, respectively) was added to the media, and the cells were incubated at 37°C for 1 h. The cells were then rapidly washed three times each with 1 ml of Opti-MEM (Invitrogen) at 37°C and were placed in 1 ml of Opti-MEM and incubated for 1 h at 37°C. The cells were visualized by phase-contrast microscopy using an Olympus IX70 microscope, and images were obtained and processed using a Olympus MagnaFire camera system.

**Tcf Reporter Gene Experiments**—Cells grown in six-well plates were transiently transfected using FuGENE 6 (Roche Applied Science) with 1 μg/well of either the TOP flash or FOP flash reporter plasmids (Upstate Biotechnology, Inc.) as described previously (5, 6). The cells were incubated at 37°C with either vehicle (0.002% sodium carbonate or 0.1% Me₂SO, final concentration, respectively) or agonist (1 μM PGF₂α or 1 μM PGE₂, final concentration, respectively) for 1 h and were rapidly washed three times each with 1 ml/well Opti-MEM and then incubated for 16 h at 37°C in 2 ml of Opti-MEM containing 250 μg/ml Geneticin, 100 μg/ml gentamicin. In the pretreatment experiments, cells were pretreated with either vehicle (0.1% Me₂SO) or various concentrations of PGE₂ for 15 min followed by the addition of vehicle (0.002% sodium carbonate) or 10 nM PGF₂α for 1 h at 37°C. Cell extracts were prepared using the Luciferase assay system (Promega). Luciferase activity was measured using a Turner TD-20/20 luminometer as described previously (5, 6) using 10 μg of protein per sample. Measurements were corrected for background activity by subtraction of the FOP flash values from the corresponding TOP flash values.

**RESULTS**

The signal transduction properties of PGF₂α and PGE₂ were characterized in untransfected HEK cells and HEK cells stably expressing either the FP_A or FP_B prostanoid receptor isoforms. Fig. 1 shows dose response curves for these prostanoids on the accumulation of total inositol phosphates. PGF₂α stimulated total inositol phosphates accumulation to similar maximal levels and with similar EC₅₀ values in both FP_A- and FP_B-expressing cells (21 nM and 16 nM, respectively). PGE₂ also stimulated total inositol phosphates accumulation to a similar maximal extent as PGF₂α, in both FP_A- and FP_B-expressing cells. However, the EC₅₀ values for PGE₂ were higher than for PGF₂α (695 nM and 117 nM, respectively). Treatment of untransfected HEK cells with PGE₂, or PGE₂ had negligible effects on total inositol phosphates formation. Thus, endogenous EP receptors that may be expressed in HEK cells contributed very little to the accumulation of inositol phosphates following treatment with PGE₂.

We have shown previously that treatment of cells expressing either the FP_A and FP_B prostanoid receptor isoforms with PGF₂α leads to Rho-mediated changes in cellular morphology consisting of the retraction of filopodia, cell rounding, and the formation of cell aggregates (3). Following the removal of PGF₂α, FP_A-expressing cells will return to their original morphology after ~1 h, whereas PGE₂-expressing cells maintain the rounded phenotype even after 24 h (4). To see if PGE₂ elicited similar effects, untransfected HEK cells and cells expressing either the FP_A or FP_B prostanoid receptors were treated with either 1 μM PGF₂α or 1 μM PGE₂ for 1 h after which they were washed and placed in fresh media. The top half of Fig. 2 shows the appearance of untransfected HEK cells and FP_A- and FP_B-expressing cells by phase-contrast microscopy after the initial 1 h treatment. As can be seen in the panels on the left, treatment of untransfected HEK cells with either PGF₂α (panels a and b) or PGE₂ (panels e and f) had no obvious effects on cell morphology. However, treatment of FP_A-expressing cells (panels a and b, center) and FP_B-expressing cells (panels a and b, right) with PGF₂α resulted in cell rounding, the formation of cell aggregates, and a loss of filopodia. Treatment of FP_A-expressing cells (panels e and f, center) and FP_B-expressing cells (panels e and f, right) with PGE₂ also produced cell rounding, the formation of cell aggregates, and a loss of filopodia that was virtually indistinguishable from the effects of PGF₂α.
The bottom half of Fig. 2 shows the appearance of cells 1 h after the removal of either PGF$_{2\alpha}$ or PGE$_2$. Panels c and g show that treatment of cells with vehicle followed by the washout procedure itself had no effect on the morphologic appearance of the cells. Thus, both the untransfected HEK cells and the FP$_{A}$- and FP$_{B}$-expressing HEK cells maintained their original morphology (compare with panels a and e). For cells expressing the FP$_{A}$, a comparison of panels c and g with panels d and h shows that following the removal of PGF$_{2\alpha}$ or PGE$_2$, there is a return to the original morphology (compare also with panels a and e). On the other hand, for cells expressing the FP$_{B}$, a comparison of panels c and g with panels d and h shows that following the removal of PGF$_{2\alpha}$, the cells still remain rounded and lack filopodia (panel d), whereas for cells initially treated with PGE$_2$ there is a return to the original morphology (panel h). Thus, the effects of PGE$_2$ in FP$_{B}$-expressing cells differ from those of PGF$_{2\alpha}$, in that the changes in cell morphology are reversible.

PGF$_{2\alpha}$-induced changes in cell morphology can be blocked by pretreatment of the cells with C3 toxin, a specific inhibitor of Rho (3). C3 toxin also inhibits the selective activation of Tcf/β-catenin signaling by the FP$_{B}$ isoform. We have hypothesized that the persistent PGF$_{2\alpha}$-induced shape change in FP$_{B}$-expressing cells may be a requirement for activation of Tcf/β-catenin signaling (6, 13). Thus, cell rounding and aggregation can be induced in FP$_{B}$-expressing cells by treatment with lysophosphatidic acid via endogenous receptors, but these effects are reversible and do not activate Tcf/β-catenin signaling (4, 6). We, therefore, used a Tcf-responsive luciferase reporter gene to investigate the potential activation of Tcf/β-catenin signaling by PGE$_2$ in FP$_{B}$-expressing cells. As shown in Fig. 3A neither PGF$_{2\alpha}$ nor PGE$_2$ had much effect on the activation of Tcf-responsive reporter gene activity in FP$_{A}$-expressing cells. In contrast, PGE$_2$ produced a marked stimulation of reporter gene activity in FP$_{B}$-expressing cells, whereas the stimulation produced by PGF$_{2\alpha}$ was much lower. It is noted that 1 μM PGE$_2$ produced nearly the same levels of total inositol phosphates as PGF$_{2\alpha}$ in FP$_{B}$-expressing cells (Fig. 1). This suggests that relative to PGF$_{2\alpha}$, PGE$_2$ is a full agonist with respect to the stimulation of inositol phosphates formation but only a partial agonist with respect to stimulation of Tcf/β-catenin signaling.

Given that both PGF$_{2\alpha}$ and PGE$_2$ are frequently up-regulated in cancer and that they can both interact with the FP receptor isoforms, we were interested in the possibility that PGE$_2$ could selectively modulate the actions of PGF$_{2\alpha}$ on the Tcf/β-catenin signaling pathway. Prior to examining this, however, we first determined the dose-response relationship for the activation of Tcf/β-catenin signaling by PGF$_{2\alpha}$. Fig. 3B shows PGF$_{2\alpha}$ dose-response curves for the activation of Tcf responsive luciferase reporter gene activity in cells expressing either the FP$_{A}$ or FP$_{B}$ receptor isoforms. It can be appreciated that the maximal activation of luciferase activity in FP$_{A}$-expressing cells was only a fraction of that obtained in FP$_{B}$-expressing cells and that maximal levels of activation were achieved with 1 μM concentrations in both cell lines. The EC$_{50}$ value for the activation of Tcf/β-catenin signaling by PGF$_{2\alpha}$, in cells expressing the FP$_{B}$ isoform was 9.6 nM. Therefore, a concentration of 10 nM PGF$_{2\alpha}$ was used for the experiments depicted in Fig. 4 that required the use of a submaximally stimulating dose of PGF$_{2\alpha}$.

In Fig. 4 the potential of PGE$_2$ to modulate the signaling activity of PGF$_{2\alpha}$ was examined in both FP$_{A}$- and FP$_{B}$-expressing cells with respect to both the activation of Tcf-responsive reporter gene activity (upper panels) and total inositol phosphates formation (lower panels). To examine this, cells were
pretreated for 15 min with concentrations of PGE₂ ranging from 0 to 10⁻⁵ M. The cells were then washed extensively and stimulated with either vehicle (closed circles) or 10 nM PGF₂α (open circles). Again, as in Fig. 3 there were negligible effects of PGF₂α on the activation of luciferase activity in FPₐ-expressing cells (top left panel). In FPₐ-expressing cells, in the absence of pretreatment with PGF₂α (0 PGE₂) stimulation, 10 nM PGF₂α increased total inositol phosphates formation by ~250% (bottom left panel). Pretreatment of FPₐ-expressing cells with 10⁻⁹ to 10⁻⁷ M PGE₂ had little effect on the stimulation of inositol phosphates formation by PGF₂α, and at concentrations of PGE₂ above 10⁻⁷ M the formation of inositol phosphates was increased because of activation of the receptor by PGE₂ itself.

On the other hand, Fig. 4 shows that in FPₐ-expressing cells the effects of pretreatment with PGE₂ on PGF₂α-activated signaling were markedly different depending upon the second messenger pathway being examined. Thus, in the absence of pretreatment with PGE₂ (0 PGE₂), 10 nM PGF₂α stimulated Tcf-responsive luciferase activity ~300% and as the concentration of PGE₂ was increased there was a marked inhibition of PGF₂α-stimulated luciferase activity (top right panel). This inhibition of luciferase activity leveled off at pretreatment concentration of ~10⁻⁸ M PGE₂, at which concentration the amount of luciferase activity was equal to that obtained by PGE₂ itself (closed circles). The EC₅₀ for PGE₂ with respect to the inhibition of PGF₂α-stimulated Tcf signaling was 98 nM, which is comparable with its EC₅₀ for the stimulation of inositol phosphates formation (117 nM, Fig. 1) suggesting that the effects of PGE₂ are mediated by direct interactions with the FPₐ receptor. Pretreatment of FPₐ-expressing cells with PGE₂, however, did not inhibit the stimulation of inositol phosphates formation by 10 nM PGF₂α (bottom right panel); thus, the pattern of activity was very similar to that observed in FPₐ-expressing cells.

**Discussion**

It has been found in a number of studies that aspirin and other nonsteroidal anti-inflammatory drugs, as well as selective COX-2 inhibitors, can attenuate the size and number of colon adenomas (11). These effects are presumably mediated through the inhibition of PG synthesis, although other mechanisms have been proposed (11). In support of a mechanism involving the PGs and their receptors is evidence from gene knock-out studies that impair EP₂ and EP₃ receptors in various mouse models of colon cancer (15, 16). In addition, we have suggested the possible involvement of an FPₐ- or FPₐ-like receptor as a factor in the development of colorectal cancer by virtue of its ability to activate the Tcf/β-catenin signaling pathway (5).

It is well established that there is an elevated expression of COX-2 and increased concentrations of PGE₂ and PGF₂α in a variety of human cancers (7, 9–11). PGE₂ and PGF₂α are structurally identical except at the C-9 position in the cyclopentane ring where PGE₂ has a keto substituent and PGF₂α has a hydroxyl. It is not unexpected, therefore, that there is some degree of cross-reactivity for PGE₂ to activate FP receptors and for PGF₂α to activate EP receptors (17). For example, based on radioligand binding PGE₂ is only about 30-fold less potent than PGF₂α at the human FP receptor (IC₅₀ values of 85 nM and 2.8 nM, respectively) (18). In the present study we explored the interactions of PGF₂α and PGE₂ with the ovine FPₐ and FPₐ alternative mRNA splice variants. We found that in cells expressing the FPₐ isoform PGE₂ can markedly inhibit PGF₂α stimulation of Tcf/β-catenin signaling without affecting PGF₂α stimulation of total inositol phosphates formation. These findings indicate that the relative composition of PGs in a tissue could be significant with respect to the activation of specific signal transduction cascades by a given prostanooid receptor subtype and its cognate ligand.

These data also lend support to thoughts about the influence of the chemical structure of agonists as it concerns the relative dominance of different conformational states of the receptor that are required for the activation of multiple signal transduction pathways (19). The most straightforward interpretation of our data with respect to the differential activation of inositol phosphates signaling as compared with Tcf/β-catenin signaling is the concept that PGF₂α and PGE₂ differ simply in terms of their relative strength to generate an initial signal by the FPₐ receptor. This interpretation does not require that the FPₐ receptor couple to different G-proteins with respect to the activation of these two signaling pathways, but we would assume this to be the case. Therefore, although it has not been specifically verified experimentally, it is likely that coupling to the inositol phosphates pathway is through the G₁₁ family of G-proteins and that coupling to Tcf/β-catenin signaling is through G₁₂/₁₃ family of G-proteins. Under these circumstances differences in initial signal strength arise from differences in the efficacy of PGF₂α or PGE₂ to cause activation of either the G₁₁ or G₁₂/₁₃ family of G-proteins. Thus, PGF₂α appears to have higher efficacy for the activation of both families of G-proteins, whereas PGE₂ has lower efficacy and preferentially promotes activation of the most efficiently coupled family of G-proteins, i.e. G₁₁.
The apparent antagonist activity of PGE$_2$ on PGF$_{2\alpha}$-mediated Tcf/\(\beta\)-catenin signaling by the FP$_B$ isoform, however, is harder to explain by simple differences in the strength of signaling. To explain this, it is possible that stimulation of the FP$_B$ receptor by PGF$_{2\alpha}$ results in selective trafficking (e.g., ternary complex formation) of the FP$_B$ receptor to both the G$_{q/11}$ and G$_{12/13}$ families of G-proteins. On the other hand, stimulation of the FP$_B$ receptor by PGE$_2$ primarily directs the receptor to the G$_{q/11}$ family of G-proteins. However, because PGE$_2$ acts as a very weak partial agonist on the Tcf/\(\beta\)-catenin pathway, it is capable of causing a functional antagonism with respect to the ability of PGF$_{2\alpha}$ to promote ternary complex formation of the FP$_B$ receptor with the G$_{12/13}$ family of G-proteins.

One additional possibility is that our findings could be explained by the activation of an endogenous EP receptor, which in the presence of FP$_B$ receptor overexpression could give rise to a unique PGE$_2$ response, possibly through receptor heterodimerization. We believe this is unlikely for several reasons. First, the data in Fig. 2 show that in untransfected HEK cells there is no cell rounding response to treatment with either 1 \(\mu\)M PGF$_{2\alpha}$ or PGE$_2$, so if there are endogenous FP or EP receptors, stimulation of these receptors alone is not sufficient to cause changes in cell morphology. Second, we have done radioligand binding with \[^{3}H\]PGE$_2$ and have examined PGE$_2$-stimulated cAMP formation in untransfected HEK cells (20), and in both cases there is little evidence for any significant response resulting from presence of an endogenous EP receptor. We are aware, however, of anecdotal reports of a PGE$_2$-induced cAMP response in some lines of HEK cells, possibly through an EP$_4$ receptor; although, as noted above we have no conclusive evidence of this in the cells that we have been using. Nevertheless, as additional control we repeated the experiments depicted in Fig. 2 with 1-hydroxy-PGE$_1$, an agonist that is relatively selective for the EP$_4$ receptor as compared with the FP receptor (21).

Treatment of untransfected HEK cells and HEK cells expressing either the FP$_A$ or FP$_B$ prostanoid receptor isoforms has also been associated with the differentiation of Caco-2 cells, a tissue culture cell line derived from human colonic carcinoma (22). Down-regulation of Tcf/\(\beta\)-catenin signaling has been associated with the transition of colorectal carcinoma cells in situ from a mesenchymal-like state to an epithelial-like state (23). Thus, well differentiated colorectal carcinomas are characterized as having two populations of cells. One population consists of differentiated epithelial-like cells with higher proliferative activity that is localized to central areas of the primary tumor and metastases. A second population consists of mesenchymal-like cells with lower proliferative activity that is localized to the invasive front of the tumor and to disseminated cells at metastatic sites. It appears that the tumor cells can undergo reversible transitions between these two states, which is somewhat at odds with a linear model of tumor progression in which the transition to a mesenchymal-like state is fixed by genetic changes (23). For example, colorectal carcinoma cells at the invasive front appear to dedifferentiate from an epithelial-like state to a mesenchymal-like state that is associated with increased metastatic potential and characterized by increased dissociation and migration. Following metastasis there appears to be redifferentiation and return to the epithelial-like condition. The epithelial-like condition is associated with de-
creased nuclear Tcf-β-catenin signaling, whereas the opposite is true for the mesenchymal-like state (23). It is believed that the regulation of Tcf/β-catenin signaling is influenced by the tumor environment, but the specific factors regulating this are not understood.

Our present findings suggest a mechanism by which the local tumor environment could alter cell signaling in the absence of transcriptional or post-transcriptional changes either in the expression of the receptors or its downstream signaling components. Thus, by relatively rapid changes in the activity of the PG biosynthetic enzymes it would be possible to change the levels of various PGs in a tissue and thereby potentially change the patterns of cell signaling. Using the FPB receptor as an example, the extent of activation of Tcf/β-catenin signaling by a fixed concentration of PGF$_2\alpha$ can be significantly reduced in the presence of PGE2 as compared with its absence. If a similar mechanism were to operate in colorectal carcinoma tumor cells, one might expect the epithelial-like condition to be associated with a relatively higher ratio of PGE2 to PGF$_2\alpha$ that would result in decreased Tcf/β-catenin signaling and the possible transition to a mesenchymal-like state. Clearly additional work will be needed to test whether such a mechanism might explain the effect of the local tumor environment on the phenotypic expression of tumor cells.

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