Prostaglandin E$_2$ Synergistically Enhances Receptor Tyrosine Kinase-dependent Signaling System in Colon Cancer Cells*

Jinyi Shao, B. Mark Evers, and Hongmiao Sheng‡

From the Department of Surgery and Sealy Center for Cancer Cell Biology, University of Texas Medical Branch, Galveston Texas 77555

Cyclooxygenase (COX)-generated prostaglandin E$_2$ (PGE$_2$) plays critical roles in colorectal carcinogenesis. Recently, we have shown that PGE$_2$ and transforming growth factor-α synergistically induces the expression of amphiregulin (AR) in colon cancer cells (Shao, J., Evers, B. M., and Sheng, H. (2003) Cancer Res. 63, 5218–5223). In this study, we demonstrated synergistic actions of PGE$_2$ and the receptor tyrosine kinase signaling system in AR expression and in tumorigenic potential of colon cancer cells. Activation of the Ras/Raf/MAPK pathway induced AR transcription in colon cancer LS-174 cells that was enhanced by PGE$_2$ in a synergistic fashion. The cAMP-responsive element within the AR promoter was required for the synergistic activation of AR transcription. An Sp1 element was responsible for the basal transcription of AR and significantly enhanced the synergy between PGE$_2$ and the epidermal growth factor receptor (EGFR) signaling system. Furthermore, activation of both PGE$_2$ and EGFR signaling pathways synergistically promoted the growth and migration of colon cancer cells. Our results suggest that COX-2/PGE$_2$ may exert pro-oncogenic effects through synergistic induction of receptor tyrosine kinase-dependent signaling pathway, thus, provide a novel mechanism for the combinatorial treatment of colonic neoplasms targeting both COX-2/PGE$_2$ and the EGFR system that has demonstrated remarkable advantages.

A large body of studies indicates that cyclooxygenase-2 (COX-2) and its derived prostaglandin E$_2$ (PGE$_2$) exert pro-oncogenic effects on colorectal neoplasms (1). Disruption of key steps of the metabolism of arachidonic acid to prostaglandins (PGs) results in tumor reduction in Apo$^{	ext{A-116}}$ mice. For examples, genetic deletion of cytosolic phospholipase A$_2$, which releases arachidonic acid from cell membrane, or knock-out of COX-2 gene, which encodes the key enzyme for conversion of arachidonic acid to PGs, results in an ~60% reduction of Apo mutation-induced intestinal adenomas in mice (2, 3). In addition, disruption of E-type prostaglandin receptor, EP$_2$, which mediates PGE$_2$ signaling, reduces the number of adenomas by ~60% in Apo$^{	ext{A-116}}$ mice as well (4), suggesting the critical role of the PGE$_2$ signaling pathway in intestinal neoplasia. PGE$_2$ promotes proliferation of human colorectal carcinoma cells (5). We have shown that PGE$_2$ stimulates the growth of human colorectal cancer cells when grown in extracellular matrix (6–8). In addition, PGE$_2$ promotes colon cancer cell migration and increases their metastatic potential (7, 9–11). PGE$_2$ acts via four PGE receptor (EP) subtypes, which are designated as EP$_1$, EP$_2$, EP$_3$, and EP$_4$ (12). EP$_2$ and EP$_4$ receptors signal through cAMP and are thought to mediate PGE$_2$ pro-oncogenic actions (4, 7, 8). Thus far, the molecular mechanisms that mediate tumor-promoting effects of the PGE$_2$ signaling system have not been extensively investigated.

The epidermal growth factor family and their cognate receptors (EGFR), referred to as the ErbB family, play critical roles in colorectal carcinogenesis (13–16). Binding of the ligand to the EGFR leads to activation of receptor tyrosine kinases (RTKs) that phosphorylate tyrosine residues of cellular signaling proteins and activate signaling pathways that are essential for intestinal epithelial proliferation and transformation (17–19). PGE$_2$ transactivates the EGFR, triggers extracellular signaling-regulated kinase (ERK) activation, and stimulates the proliferation of colorectal carcinoma cells (5, 20). The phosphatidylinositol 3-kinase Akt pathway transmits signals from tyrosine kinase-coupled receptors and plays critical roles in mitogenic signaling (21). Phosphatidylinositol 3-kinase activity that is critical for the proliferation and transformation of intestinal epithelial cells (16, 22) is required for the growth stimulation of PGE$_2$ in human colon cancer cells (7). PGE$_2$-induced DNA synthesis can be blocked by specific EGFR antagonists or antibodies to transforming growth factor α (TGF-α) and amphiregulin (5, 8). Recently, we have reported that PGE$_2$ induces the expression of amphiregulin (AR), a member of the epidermal growth factor family, which exerts mitogenic effect to colon cancer cells through an autocrine mechanism (8). We have demonstrated that PGE$_2$ and TGF-α induced the expression of AR mRNA in a synergistic manner. These studies indicate the complexity of interaction between PGE$_2$ and the EGFR signaling system and suggest that PGE$_2$ may activate RTK-dependent signaling pathways through a variety of mechanisms.

COX inhibitors are being developed as agents for the intervention of colorectal cancers (1). Elucidating the interaction between the PGE$_2$ signaling pathway and other oncogenic signaling systems further will help to design novel strategies for prevention and treatment of colon cancers. In this study, we elucidated the mechanism by which PGE$_2$ and the EGFR/Ras/MAPK system synergistically induced the expression of pro-oncogenic gene, AR. The functional synergy of PGE$_2$ and the EGFR was elucidated. PGE$_2$ and TGF-α stimulated the growth and migration of colon cancer cells in a synergistic manner. Thus, our results provide a novel mechanism for combined
treatment of colorectal neoplasia by targeting both the COX-2/ PGE2 pathway and the RTK signaling system.

MATERIALS AND METHODS

Cell Culture and Chemicals—LS-174 and T-84 human colon cancer cell lines were purchased from ATCC (Manassas, VA). LS-174 cells were maintained in McCoy’s 5A medium containing 10% fetal bovine serum. T-84 cells were maintained in Dulbecco’s modified Eagle’s medium/F12 medium containing 5% fetal bovine serum. Growth of cells in Matrigel® (Collaborative Biomedical, Bedford, MA) was carried out as described previously (7). PGE2 was purchased from Cayman Chemical (Ann Arbor, MI). TGF-β1 was purchased from R&D system (Minneapolis, MN). H-89 and PD-153035 were purchased from Calbiochem. Dibutyryl cAMP was purchased from Sigma.

Cell Migration Assays—Cell migration assay was carried out using Transwell chambers (8 μm, Corning Costar Co. Cambridge, MA). 5 × 10⁴ cells suspended in 400 μl of serum-free McCoy’s 5A medium were placed in the upper chamber. The lower chamber was filled with 1 ml of McCoy’s 5A medium containing vehicle or indicated treatment. After an incubation period of 24 h at 37 °C, the cells on the upper surface of the filter were removed with a cotton swab. The filters were fixed and stained with 0.5% crystal violet solution. Cells adhering to the underside of the filter were counted.

Transient Transfection and Luciferase Assay—The assays to determine the activity of the AR promoter were described previously (8). Reporter constructs pGL2-A, pGL2-B, pGL2-BΔCRE, pGL2-C, and pGL2-CΔCRE containing the 5'-flanking region of the human AR gene were described previously (8, 24). pGL2-A1 (417 to 192) and pGL2-A2 (372 to 192) were constructed using PCR. pGL2-A2ΔCRE was generated by digestion of pGL2-A2 with AatII and treatment with T4 DNA polymerase. For transient transfections, cells were co-transfected with indicated plasmids including 3 ng of the pRL-SV40 plasmid containing the Renilla luciferase gene (Promega, Madison WI) using the FuGENE 6 procedure (Roche Applied Science). Firefly and Renilla luciferase activities were measured, and firefly luciferase values were standardized to Renilla values (RLUs). Plotted is the mean ± S.D. of RLUs performed in quadruplicate. *p < 0.05. Results were similar in three independent experiments.

FIG. 1. Synergy between PGE2 and the Ras/Raf/MAPK pathway. A, synergy between PGE2 and oncogenic Raf. LS-174 cells were transiently co-transfected with pGL2-A plus vector, active Raf (RafBXB), or kinase dead Raf (RafKD) expression vector. PGE2 was added 6 h prior to harvest. Firefly and Renilla luciferase activities were measured, and firefly luciferase values were standardized to Renilla values (relative light units, RLUs). Plotted is the mean ± S.D. of RLUs performed in quadruplicate. Results were similar in three independent experiments. B, synergy between PGE2 and MEK1. LS-174 cells were transiently co-transfected with pGL2-A plus vector, active MEK1(SD), or wild type MEK1(WT) expression vectors. PGE2 was added 6 h prior to harvest. Firefly and Renilla luciferase activities were measured, and firefly luciferase values were standardized to Renilla values (RLUs). Plotted is the mean ± S.D. of RLUs performed in quadruplicate. C, PKA and RTK in AR promoter activation. LS-174 cells were transiently transfected with pGL2-A and subjected to the indicated treatments. V, vehicle; E2, 0.1 μM PGE2; α = 100 ng/ml TGF-α; H89, 10 μM H-89; PD, 1 μM PD-135033. Firefly and Renilla luciferase activities were measured, and firefly luciferase values were standardized to Renilla values. Plotted is the mean ± S.D. of RLUs performed in quadruplicate. *, p < 0.05. Results were similar in three independent experiments.
Fig. 2. Roles of the Sp1 domain in AR transcription. A, progressive deletion constructs of the AR promoter. LS-174 cells were transiently transfected with AR report constructs pGL2-A, pGL2-A1, pGL2-A2, pGL2-B, pGL2-C, or empty pGL2 (V). Firefly and Renilla luciferase activities were measured after a 48-h incubation, and firefly luciferase values were standardized to Renilla values (relative light units, RLUs). Plotted is the mean ± S.D. of RLUs performed in quadruplicate. *, p < 0.05. Results shown are representative of three separate experiments. Schematic representation of human AR promoter constructs was shown in the bottom panel. Luc, luciferase. B, PGE2, and TGF-α synergy in pGL2-A2 activity. LS-174 cells were transiently transfected with pGL2-A2, which contains a Sp1, a CRE, and a TATA box. Cells were then treated as indicated (V, vehicle; $E_2$, 0.1 μM PGE2; $α = 100$ ng/ml TGF-α) for 6 h. Firefly and Renilla luciferase activities were measured and standardized (RLUs). Plotted is the mean ± S.D. of RLUs performed in quadruplicate. Results shown are representative of five separate experiments.

**RESULTS**

Synergy between PGE2 and the Ras/Raf/MAPK Pathway—Cell growth and transformation require the expression of a subset of pro-oncogenic genes. Amphiregulin plays critical roles in colon cancer cell proliferation and transformation (25, 26). We have demonstrated that PGE2 and TGF-α increase the expression of AR mRNA through synergistic induction of AR transcription (8). The Ras/Raf/MAPK pathway plays a central role in transduction of signals from EGFR. PGE2 and oncogenic K-Ras induces AR transcription in a synergistic manner (8). To completely assess the interaction between PGE2 and the Ras/Raf/MAPK pathway, we ectopically introduced mutated Raf and MAPK/ERK kinase (MEK) into LS-174 cells. Transfection with an active Raf expression vector, Raf-BXB (16), increased AR transcription ~2-fold. The addition of PGE2 resulted in an ~2-fold increase in the activity of AR promoter when compared with the cells transfected with empty vector (Fig. 1A). In contrast, expression of a kinase-dead Raf construct did not change AR transcription. Moreover, expression of an active form of MEK1 increased AR promoter activity ~13-fold. Again, a synergistic induction was observed between PGE2 and MEK1 activity (Fig. 1B). These results suggest a synergy between PGE2 signaling and the Ras/Raf/MAPK pathway in context of AR transcription.

TGF-α signals through the EGFR and RTK-dependent signaling pathways. PGE2 acts through induction of cAMP and activation of protein kinase A (PKA). Inhibition of either EGFR tyrosine kinases or the cAMP/PKA pathway attenuated the synergy of TGF-α and PGE2. Either a selective PKA inhibitor, H-89, or a specific EGFR tyrosine kinase inhibitor, PD-153055, partially attenuated the synergistic action of PGE2 and TGF-α. Combined treatment with H-89 and PD-153055 completely blocked the induction of AR transcription by PGE2 and TGF-α (Fig. 1C).

Synergy between PGE2 and TGF-α Synergy—Human AR promoter contains a number of functional domains, as schematically demonstrated in Fig. 2A (lower panel). To determine the relative contribution of each transcriptional element in AR transcription, progressive deletion constructs were generated. The luciferase activity observed with reporter vectors pGL2-A, pGL2-A1, and pGL2-A2 was approximately 20 times higher than that with pGL2 vector (Fig. 2A, upper panel). The major promoter activity was mapped to 44 nucleotides between −372 and −328 where an Sp1 consensus sequence (−353 to −348) was identified. Removal of the Sp1 site dramatically reduced AR promoter activity. Luciferase activity was decreased by ~92% in pGL2-B-transfected cells. The reporter vector pGL2-A2, which contains the AR promoter sequence from −372 to −192 including the Sp1, a Wilms’ tumor suppressor WT1-responsive element consensus, a CRE, and a TATA box, was constructed. PGE2 and TGF-α synergistically induced the luciferase activity in pGL2-A2-transfected LS-174 cells, suggesting these cis-elements are sufficient for the PGE2 and TGF-α synergy on AR transcription (Fig. 2B).

Critical Roles of CRE in the PGE2 and TGF-α Synergy—The CRE element within AR promoter is critical for PGE2 induction of AR transcription (8). To evaluate the role of the Sp1 and the CRE in PGE2/TGF-α synergistic induction of AR transcription, the CRE element was mutated in pGL2-A2 construct and referred to as pGL2-A2CRE. Although the basal activity of pGL2-A2 and pGL2-A2CRE was similar, PGE2 did not increase the luciferase activity in pGL2-A2CRE-transfected LS-174 cells. The synergistic effect of PGE2 and TGF-α was also attenuated by disruption of the CRE (Fig. 3A). Furthermore, induction of AR transcription by oncogenic K-Ras required the CRE domain. The synergistic induction of AR transcription by oncogenic K-Ras and PGE2 was attenuated when LS-174 cells
were transiently transfected with pGL2-A2 CRE reporter construct (Fig. 3B). To confirm the critical role of the CRE in AR transcription, we co-transfected LS-174 cells with a wild type CREB expression vector and pGL2-A reporter vector. Expression of wild type CREB increased the level of luciferase activity 9-fold (Fig. 3C). In contrast, expression of a dominant negative form of CREB reduced the basal AR transcription and significantly blocked PGE2 activation of the AR promoter as well. In agreement with these observations, the addition of cAMP significantly increased AR promoter activity. A synergistic induction of AR transcription was noted when LS-174 cells were treated with pGL2-A or co-transfected with pGL2-A plus MEK1(SD) expression vector. Cells were subjected to the indicated treatments (cAMP, 0.5 mM dibutyryl camp; α = 100 ng/ml TGF-α) for 6 h. Firefly and Renilla luciferase activities were measured and standardized (RLUs). Plotted is the mean ± S.D. of RLUs performed in quadruplicate. Results shown are representative of three separate experiments.

We have demonstrated that PGE2 treatment rapidly induces the phosphorylation of CREB at Ser-133. Several studies indicate that TGF-α may induce phosphorylation and activation of CREB through the ERK/p90 Rsk pathway (28, 29). Western analysis revealed that TGF-α treatment increased levels of phosphorylated ERK, phosphorylated Rsk, and phosphorylated CREB (Ser-133) in LS-174 cells (Fig. 4C).
Cell Migration

Several studies have demonstrated that PGE2—

Cell Growth—Because the EGFR signaling system and the
targeting effects on LS-174 cells (Fig. 5B). Cells were then treated (V, vehicle; E2, 0.1 μM PGE2; α, 100 ng/ml TGF-α) for
6 h. Firefly and Renilla luciferase activities were measured and standardized (relative light units, RLU). Plotted is the
mean ± S.D. of RLUs performed in quadruplicate. Results shown are representative of five separate experiments. C, TGF-α activation of the ERK/Rsk/CREB pathway. Serum-deprived LS-174 cells were treated with TGF-α for the indicated times. Cellular protein was analyzed for the levels of pERK, pRsk, and pCREB by Western blot.

PGE2 and TGF-α Synergistically Stimulated Colon Cancer Cell Growth—Because the EGFR signaling system and the
COX-2/PGE2 signaling system can synergistically induce pro-
oncogenic gene expression, it was of interest to determine whether these two signaling systems collaboratively enhance
tumorigenic potential. We first elucidated the growth regulatory effects of PGE2 and TGF-α in colon cancer cells. LS-174
cells are able to form “tumor-like” aggregates when they are cultured in Matrigel (7). The colonies consisted of several layers
of cells and a mucin-filled center. Morphologically, colonies formed by LS-174 cells in Matrigel were highly similar to
LS-174 xenografts in athymic nude mice (Fig. 5A). The addition of TGF-α or PGE2 increased the size of colonies. In combination,
TGF-α and PGE2 synergistically exerted growth-stimulatory
effects on LS-174 cells (Fig. 5B). Colon cancer T-84 cells, which have been reported to react to PGE2 treatment (30),
formed similar three-dimensional structures in Matrigel. The
synergistic stimulation of TGF-α and PGE2 on cell growth was
observed in T-84 cells as well (Fig. 5C). These results suggest that TGF-α and PGE2 may stimulate the growth of colon tumors in a synergistic manner.

PGE2 and TGF-α Synergistically Stimulated Colon Cancer Cell Migration—Several studies have demonstrated that PGE2 promotes migration and invasion of colon cancer cells (7, 10, 11). On the other hand, TGF-α-activated EGFR signaling is critical for colon cancer cell migration (31). To elucidate the collaborative regulation of cell migration by PGE2 and TGF-α, LS-174 cells were subjected to the Modified Boyden chamber assay (Transwell). Vehicle-treated LS-174 cells formed non-invasive clumps on the membrane after a 24-h incubation, so that most micropores were not covered by cells. LS-174 cells strongly spread when both PGE2 and TGF-α were present in the bottom chamber (data not shown). As a result, PGE2 and TGF-α synergistically stimulated LS-174 cell migration (Fig. 6A) and increased the number of cells that penetrated the

DISCUSSION

Colorectal cancers typically develop over decades and require a number of genetic changes for completion (32). Epidemiological studies demonstrate a 40–50% reduction in the relative risk of colorectal cancer and colorectal cancer-associated mortality in individuals taking nonsteroidal anti-inflammatory
drugs on a regular basis (33–35). Genetic disruption of the
COX-2/PGE2 pathway results in a ~60% tumor reduction in
Apc+/- mice (2, 4). These observations indicate the critical role of COX-2/PGE2 in colon cancer development and also raised an important question. What is the molecular mechanism that mediates the chronic pro-oncogenic action of the COX-2/PGE2 signaling pathway in colorectal neoplasia? Recent studies have demonstrated that PGE2 induces rapid phosphorylation and transactivation of the EGFR through Src-mediated signaling (5, 10, 11). Transactivation of the EGFR is particularly critical for PGE2-induced growth and invasion of colon cancer cells. Colorectal neoplasms are frequently associated with alterations of the EGFR signaling system. Epidermal growth factor receptors including their non-ligand-binding co-receptor, ErbB-2, are often up-regulated in colorectal neoplasms (36, 37). Overexpression of EGFR ligands including TGF-α, amphiregulin, and cripto in colonic tumors is well documented (25, 36, 38). Transactivation of the EGFR by COX-generated PGE2 may further increase the activity of the EGFR and, therefore, enhance the oncogenicity of the EGFR signaling system. However, additional mechanisms appear to be necessary for the understanding of the key roles of COX-2/PGE2 in colorectal neoplasia that have been observed in epidemiological studies and in animal experiments as well (2, 4, 33–35). Our results show that the COX-2/PGE2 signaling pathway and the RTK-dependent signaling system promoted the growth and migra-
These experiments were repeated three times.

PGE2 may dramatically increase the activity of major onco-naling pathway; however, our results suggest that COX-2/ured. Relative colony size to the control group is plotted. *, p

Raf/MEK/ERK pathway at different levels and thereby regu-
lation of colon cancer cells in a synergistic fashion. These two signaling systems synergistically induced the expression of AR, providing the evidence that COX-2/PGE2 may collaboratively enhance the expression of pro-oncogenic genes, which are regulated by growth factors and oncogenes and which are critical for neoplastic transformation of intestinal epithelium. Thus far, COX-2/PGE2 itself is not considered as an oncogenic signal-

Cumulative evidence suggests that COX-2 is a target gene of oncogenic Ras (39–41). Inhibition of COX-2 enzyme activity suppresses the growth of xenografts of Ras-transformed rat intestinal epithelial cells in nude mice, suggesting the involve-
ment of COX-2 in Ras-mediated transformation (42). We have reported that COX-2-generated prostacyclin enhances Ras-me-
diated activation of peroxisome proliferator-activated receptor δ (43), which has been shown to protect colon cancer cells from apoptosis (44). In this study, we found that PGE2 synergisti-
cally enhanced Ras-induced transcription of a pro-oncogenic gene, AR, of which overexpression and involvement in colorec-
tal tumors are well investigated (25, 38). These findings sug-

The serine/threonine kinase Raf is a downstream effector of Ras (45). Activation of Raf is necessary and sufficient for the activation of the MEK/ERK cascade. Activated ERK phosphor-
ylates and activates the Elk-1 transcription factor, thereby stimu-
lating the transcription of genes controlled by the serum response element (46). The Raf/MEK/ERK pathway plays an important role in the control of metabolic processes, cell cycle, cell migration, cell proliferation, and cell transformation (47) and can be activated by a large number of growth factors and hormones through Ras-dependent or Ras-independent mecha-
nisms (48). Our results demonstrate a direct collaboration among PGE2, Raf and PGE2, and MEK1 in regulation of AR transcription, suggesting that PGE2 is able to modulate the Raf/MEK/ERK pathway at different levels and thereby regulate a wide variety of cellular functions.

The human AR promoter contains a number of functional domains (24, 27). We demonstrate that the activation of the AR
promoter was controlled by a constitutive and a regulatory domain. The Sp1 element appeared to provide the constitutive activity of the AR promoter, whereas the CRE was responsible for the regulatory activation of the promoter. Deletion of the Sp1 sequence reduced AR promoter activity by 90% but preserved its reactivity to PGE2 and TGF-α stimulation. In contrast, disruption of the CRE domain maintained the basal activity of AR promoter but attenuated the PGE2/TGF-α-induced AR transcription. Our data suggest that the cAMP/PKA pathway clearly mediated PGE2 signaling and was required for PGE2 induction of AR transcription. On the other hand, the effect of TGF-α on AR transcription was modest. TGF-α treatment resulted in phosphorylation of ERK, Rsk, and CREB, suggesting that TGF-α may regulate AR transcription via CRE as well (28, 29). Additional experiments are required to determine the precise mechanism by which PGE2 and TGF-α synergistically induce AR transcription.

Combination of anti-cancer agents that target several genetic or biochemical alterations involved in neoplasms may result in better therapeutic response than single treatment. Torrance et al. (49) report that a combination of a nonsteroidal anti-inflammatory drug, sulindac, and an EGFR kinase inhibitor, EKI-569, achieved remarkable protection from intestinal neoplasia in ApcMin/+ mice. Although untreated ApcMin/+ mice developed ~20 polyps/mouse, EKI-569 alone reduced intestinal polyps by 50% and sulindac alone inhibited the number of polyps by 70%. Interestingly, nearly half of the mice treated with these two agents developed no polyps at all. In another study (50), combined therapy with a selective COX-2 inhibitor, celecoxib, and an antibody to Her-2/neu, a member of the ErbB/Her family of growth factor receptors, results in almost complete inhibition of the growth of xenografted colon cancers. The mechanisms by which these combinatorial treatments produce remarkable results were not clear, and choosing these therapeutic mechanisms by which these combinatorial treatments produce complete inhibition of the growth of xenografted colon cancers. The study (50), combined therapy with a selective COX-2 inhibitor, celecoxib, and an antibody to Her-2/neu, a member of the ErbB/Her family of growth factor receptors, results in almost complete inhibition of the growth of xenografted colon cancers. The mechanisms by which these combinatorial treatments produce remarkable results were not clear, and choosing these therapeutic mechanisms was based on the individual activity of genes involved in signaling pathways. Our studies suggest that COX-2/PGE2 and the EGFR signaling system synergistically induce the expression of critical genes for colonic neoplasms and, therefore, provide a potential mechanism for the combinatorial treatment of colonic neoplasia targeting both the COX-2/PGE2 signaling pathway and the EGFR signaling system that has demonstrated significant advantages.

Acknowledgments—We thank Dr. Sean B. Lee (NIDDK, National Institutes of Health) for providing amphiregulin promoter constructs and Dr. Michael White (University of Texas Southwestern Medical Center, Dallas, TX) for providing Raf expression construct.

REFERENCES
1. Gupta, R. A., and Dubois, R. N. (2001) Nat. Rev. Cancer 1, 11–21
2. Oshima, M., Dinchuk, J. E., Kargman, S., Oshima, H., Hancock, B., Kwon, E., Trezaskos, J. M., Evans, J. F., and Taketo, M. M. (2000) J. Biol. Chem. 275, 34013–34016
3. Sonoshita, M., Sasaki, N., Uozumi, N., Doi, Y., Shimizu, T., and Taketo, M. M. (2000) J. Biol. Chem. 275, 34013–34016
4. Sheng, H., Shao, J., and Dubois, R. N. (2001) J. Biol. Chem. 276, 18075–18081
5. Sheng, H., Shao, J., and Dubois, R. N. (2001) Cancer Res. 61, 2670–2675
6. Scheng, G. G., Shao, J., Hooton, E. B., Isakson, P. C., Morrow, J. D., Coffee, R. J., and Dubois, R. N. (2002) Cancer Res. 62, 3288–3288
7. Scheng, G. G., Shao, J., Hooton, E. B., Isakson, P. C., Morrow, J. D., Coffee, R. J., and Dubois, R. N. (2002) Cancer Res. 62, 3288–3288
8. He, T. C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. (1999) Cell 99, 335–345
9. Campbell, S. L., Kosorov-Far, B., Rossman, K. L., Clark, G. J., and Der, C. J. (1998) Oncogene 17, 1395–1413
10. Gille, H., Kortenjann, M., Thomas, O., Moewes, C., Caffer, C., Cobb, M. H., and Sho, P. E. (1995) EMBO J. 14, 951–962
11. Schlessinger, J. (2000) Cell 103, 211–225
12. Peyssonnaux, C., and Eychene, A. (2001) Cell 93, 63–66
13. Terrance, C. J., Jackson, P. E., Montgomery, E., Kinzler, K. W., Vogelstein, B., Grimer, A., and Dicafani, C. M. (2000) Nat. Med. 6, 1024–1028
14. Manno, M., Sheng, H., Shao, J., Williams, C. S., Puscas, P. I., Slawikowski, M. X., and Dubois, R. N. (2001) Gastroenterology 120, 1713–1719

Downloaded from http://www.jbc.org/ by guest on July 21, 2018
Prostaglandin E$_2$ Synergistically Enhances Receptor Tyrosine Kinase-dependent Signaling System in Colon Cancer Cells
Jinyi Shao, B. Mark Evers and Hongmiao Sheng

J. Biol. Chem. 2004, 279:14287-14293.
doi: 10.1074/jbc.M313276200 originally published online January 23, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M313276200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 19 of which can be accessed free at http://www.jbc.org/content/279/14/14287.full.html#ref-list-1