

# Vascular Endothelial Growth Factor Gene Expression in Colon Cancer Cells Exposed to Prostaglandin E<sub>2</sub> Is Mediated by Hypoxia-inducible Factor 1<sup>1</sup>

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## ABSTRACT

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) has been implicated as an inducer of angiogenesis in human colon cancer. Here, we demonstrate that PGE<sub>2</sub> exposure induces the expression of vascular endothelial growth factor (VEGF) mRNA in HCT116 human colon carcinoma cells that is mediated by the transcriptional activator hypoxia-inducible factor 1 (HIF-1). PGE<sub>2</sub> exposure induces the phosphorylation of extracellular signal-regulated kinase (ERK) and AKT. Pharmacologic inhibition of ERK phosphorylation blocks the induction of VEGF mRNA and HIF-1 $\alpha$  protein expression in response to PGE<sub>2</sub> stimulation. Inhibition of C-SRC tyrosine kinase activity also blocks PGE<sub>2</sub>-induced HIF-1 $\alpha$  protein and VEGF mRNA expression without blocking ERK phosphorylation. In contrast, phosphorylation of AKT is dependent on ERK and C-SRC activity. Thus, the activity of multiple signal transduction pathways is required for the HIF-1-mediated induction of VEGF expression in colon cancer cells exposed to PGE<sub>2</sub>.

## INTRODUCTION

Colorectal cancer is the second leading cause of mortality from neoplastic disease in the United States. Tumor microvessel density is an important determinant of colon cancer metastasis and patient mortality (1, 2). The level of VEGF<sup>3</sup> expression is a major determinant of microvessel density in colon cancers, and VEGF expression is also correlated with metastasis and mortality. Recent studies of human colon cancers have demonstrated that expression of COX2 is also correlated with VEGF expression, angiogenesis, and patient mortality (3–5). COX2 catalyzes the production of PGE<sub>2</sub>, and high levels of PGE<sub>2</sub> have been demonstrated in colorectal adenocarcinomas in comparison with adjacent normal tissue (6, 7). In mice, pharmacologic or genetic inhibition of COX2 activity suppresses colon carcinogenesis (8–10) and can block the growth of established tumors (11). In humans, the use of nonsteroidal anti-inflammatory drugs that nonselectively inhibit COX2 is associated with a reduced risk of colorectal cancer (12–15).

The mechanism by which COX2-generated PGE<sub>2</sub> induces VEGF expression in colon cancer cells has not been determined. VEGF expression is also induced in colon cancer cells by other stimuli, including hypoxia, activation of the IGF-1R, or p53 loss-of-function, and, in these cases, transcriptional activation of the *VEGF* gene is mediated by HIF-1 (16, 17). HIF-1 is a heterodimeric protein consisting of a constitutively expressed HIF-1 $\beta$  subunit and a HIF-1 $\alpha$  subunit, the expression of which is regulated by the cellular O<sub>2</sub> concentration and diverse signal transduction pathways leading from cell surface receptors to kinase cascades (18). Under hypoxic condi-

tions, the O<sub>2</sub>-dependent hydroxylation of proline residues 402 and 564, which is required for ubiquitination and proteasomal degradation of HIF-1 $\alpha$ , is inhibited (19–21). In contrast, activation of the PI3K and ERK pathways after ligand binding to the IGF-1R stimulates increased synthesis of HIF-1 $\alpha$  protein (16). HIF-1 $\alpha$  overexpression has been demonstrated in human colon cancer biopsies (22, 23), and forced overexpression of HIF-1 $\alpha$  in human colon carcinoma HCT116 cells increases tumor growth and angiogenesis in nude mice (17).

In the present study, we investigated the mechanism by which PGE<sub>2</sub> exposure induces *VEGF* gene expression in HCT116 human colon carcinoma cells. We demonstrate that PGE<sub>2</sub> induces expression of HIF-1 $\alpha$  protein and VEGF mRNA and that inhibition of HIF-1 $\alpha$  expression by RNA interference blocks the induction of VEGF mRNA. We also provide evidence that the activity of multiple signal transduction pathways is required for the HIF-1-mediated induction of VEGF expression in colon cancer cells exposed to PGE<sub>2</sub>.

## MATERIALS AND METHODS

**Tissue Culture and Reagents.** HCT116 cells (wild type with respect to p53 expression) were cultured in McCoy's 5A medium with 10% FBS, 100 units/ml penicillin, and 100  $\mu$ g/ml streptomycin (Invitrogen Corp., Carlsbad, CA). PGE<sub>2</sub>, PD98059, Wortmannin, rapamycin, SB203580, cycloheximide, and cobalt chloride (CoCl<sub>2</sub>) were from Sigma (St. Louis, MO). SC-51322 and 17-pt-PGE<sub>2</sub> were from Biomol Research Laboratories (Butler Pike, PA). PP2 and JNK inhibitor were from Calbiochem. For hypoxic exposures, cells were placed in a modulator incubator chamber (Billups-Rothenberg, Del Mar, CA) flushed with 1% O<sub>2</sub>/5% CO<sub>2</sub>/balance N<sub>2</sub>, sealed, and incubated at 37°C.

**PGE<sub>2</sub> and Inhibitor Treatments.** HCT116 cells were plated at a density of 2.5  $\times$  10<sup>6</sup>/10-cm or 8.6  $\times$  10<sup>5</sup>/6-cm dish. Subconfluent cells were serum starved (0.1% FBS) for 24 h before PGE<sub>2</sub> or 17-pt-PGE<sub>2</sub> was added. Kinase inhibitors PD98059, Wortmannin, rapamycin, and SB203580 were added 1 h before exposure to PGE<sub>2</sub>, 1% O<sub>2</sub>, or 100  $\mu$ M CoCl<sub>2</sub>. JNK inhibitor and PP2 were added 20 min and 2 h, respectively, before the exposure to PGE<sub>2</sub> or 1% O<sub>2</sub>. EP<sub>1</sub> receptor antagonist SC-51322 was added 30 min before exposure to PGE<sub>2</sub>. Cycloheximide was added to the media of HCT116 cells that had been serum starved and treated with CoCl<sub>2</sub> for 4 h or PGE<sub>2</sub> for 24 h, and whole cell extracts were prepared at 0, 20, and 40 min.

**Immunoblot Assays.** Whole cell extracts were prepared using radioimmunoprecipitation assay buffer, fractionated by SDS-PAGE, and transferred to a nitrocellulose filter. For HIF-1 $\alpha$  and HIF-1 $\beta$ , 150- $\mu$ g aliquots of protein were analyzed using a monoclonal antibody against HIF-1 $\alpha$  (H1 $\alpha$ 67; Ref. 23) or HIF-1 $\beta$  (H1 $\beta$ 234; Novus Biologicals, Littleton, CO; Ref. 24) at 1:1000 dilution (16). Aliquots (50  $\mu$ g) were analyzed using antibodies (1:1000 dilution) specific for phosphorylated (Thr202/Tyr204) or total p44/p42 MAP kinase and phosphorylated (Ser473) or total AKT (Cell Signaling Technology, Beverly, MA, and Santa Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase-conjugated secondary antibodies against mouse and rabbit IgG (1:2500 dilution) and enhanced chemiluminescence reagents were from Amersham Biosciences (Piscataway, NJ).

**RT-PCR Assays.** Total RNA was extracted from HCT116 cells using TRIzol reagent (Invitrogen Corp.). Aliquots (5  $\mu$ g) of RNA were reverse transcribed to cDNA using Superscribe First-Strand Synthesis System (Invitrogen Corp.). Aliquots (1, 2, and 4  $\mu$ l) of cDNA were used as template for PCR of HIF-1 $\alpha$ , VEGF, and 18S rRNA sequences. The following oligonucleotides were used as primers: (a) 5'-GGGAGAAAATCAAGTCGTGC-3' and 5'-AGCAAGGAGGGCCTCTGATG-3' (HIF-1 $\alpha$ ); (b) 5'-TACCTCCACCAT-GCCAAGTG-3' and 5'-AAGATGTCCACCAGGGTCTC-3 (VEGF); and (c) 5'-ATCCTGCCAGTAGCATATGC-3' and 5'-ACCGGGTTGGTTTGTAT-

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<sup>3</sup> The abbreviations used are: VEGF, vascular endothelial growth factor; IGF, insulin-like growth factor; ERK, extracellular signal-regulated kinase; COX2, cyclooxygenase 2; HIF-1, hypoxia-inducible factor 1; PI3K, phosphatidylinositol-3-kinase; 17-pt-PGE<sub>2</sub>, 17-phenyl-trinor-prostaglandin E<sub>2</sub>; IGF-1R, insulin-like growth factor-1 receptor; MEK, mitogen-activated protein/extracellular signal-regulated kinase kinase; JNK, c-Jun NH<sub>2</sub>-terminal kinase; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; MAP, mitogen-activated protein; RT-PCR, reverse transcription-PCR.

CTG-3' (18S rRNA). Thermocycling conditions were 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C for 25 (HIF-1 $\alpha$ ), 27 (VEGF), or 13 (18S rRNA) cycles.

**RNA Interference.** To generate siRNA<sub>HIF-1 $\alpha$</sub> , two oligonucleotides consisting of ribonucleosides, except for the presence of 2'-deoxyribonucleosides at the 3' end (5'-AGAGGUGGAUAUGUGGGdTdT-3' and 5'-CCCACA-CAUACCACCUCUdTdT-3'), were synthesized and annealed (Dharmacon Research, Inc., Lafayette, CO). HCT116 cells were plated at  $2 \times 10^6$  cells/10-cm dish and exposed to 100 nM siRNA<sub>HIF-1 $\alpha$</sub>  in the presence of Oligofectamine (Invitrogen Corp.) for 4 h and then cultured for 24 h in complete media (25). Cells were serum starved for another 24 h and exposed to 100  $\mu$ M PGE<sub>2</sub> or vehicle for 24 h, and total RNA was isolated for RT-PCR analysis of HIF-1 $\alpha$  and VEGF mRNA. As control, cells were exposed to Oligofectamine without siRNA<sub>HIF-1 $\alpha$</sub> . Neither mock transfection nor transfection with an siRNA targeted to an irrelevant mRNA inhibited HIF-1 $\alpha$  mRNA or protein expression (26).

## RESULTS

We first performed a time course experiment in which HCT116 cells were serum starved and exposed to PGE<sub>2</sub> for 0–40 h before preparation of whole cell protein lysates or isolation of total RNA. Immunoblot assay revealed that HIF-1 $\beta$  protein was constitutively expressed and HIF-1 $\alpha$  protein expression was induced with peak levels observed 18–32 h after the addition of PGE<sub>2</sub> to the culture media (Fig. 1A). In contrast, we have demonstrated previously that maximal induction of HIF-1 $\alpha$  protein expression is observed 8 h after IGF-1 addition to HCT116 cells (16). Thus, compared with IGF-1, PGE<sub>2</sub> induces HIF-1 $\alpha$  protein expression with delayed kinetics. HIF-1 $\alpha$  mRNA expression was not induced by PGE<sub>2</sub> stimulation (Fig. 1A), indicating that the increased HIF-1 $\alpha$  protein levels resulted from either increased protein synthesis or decreased protein degradation. VEGF mRNA expression was induced by PGE<sub>2</sub> with kinetics similar to those observed for HIF-1 $\alpha$  protein expression (Fig. 1A). Exposure of HCT116 cells to various concentrations of PGE<sub>2</sub> for 24 h induced HIF-1 $\alpha$  protein and VEGF mRNA expression in a dose-dependent manner, although the responses were modest in comparison with the responses induced by hypoxia (Fig. 1B).

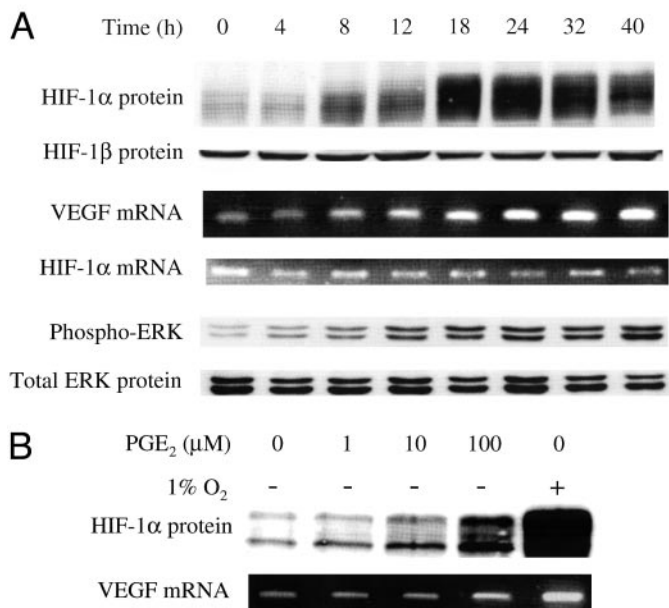


Fig. 1. PGE<sub>2</sub>-induced responses in HCT116 cells. **A**, time course. Cells were serum starved for 24 h; 100  $\mu$ M PGE<sub>2</sub> was added to the media for the indicated time, and cells were harvested for immunoblot (HIF-1 $\alpha$ , HIF-1 $\beta$ , phospho-ERK [Thr202/Tyr204], and total ERK protein) and RT-PCR (VEGF and HIF-1 $\alpha$  mRNA) assays. **B**, dose response. Cells were untreated (Lane 1) or exposed to 1–100  $\mu$ M PGE<sub>2</sub> (Lanes 2–4) or 1% O<sub>2</sub> (Lane 5) for 24 h and harvested for analysis of HIF-1 $\alpha$  protein or VEGF mRNA.

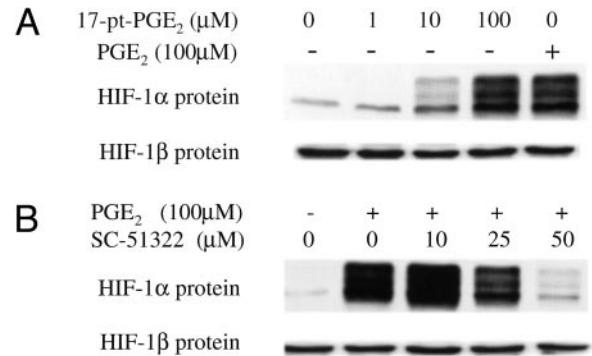


Fig. 2. Effect of EP<sub>1</sub> receptor agonist and antagonist. In **A**, HCT116 cells were untreated (Lane 1) or exposed to the EP<sub>1</sub> receptor agonist 17-pt-PGE<sub>2</sub> (Lanes 2–4) or PGE<sub>2</sub> (Lane 5) for 24 h. In **B**, cells were untreated (Lane 1) or exposed to PGE<sub>2</sub> for 24 h in the presence of 0–50  $\mu$ M EP<sub>1</sub> receptor antagonist SC-51322 (Lanes 2–5).

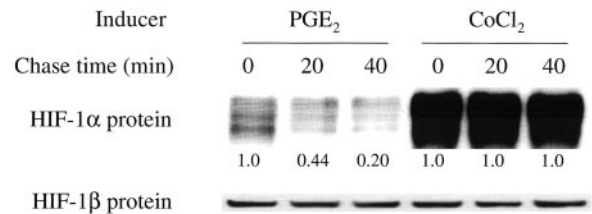


Fig. 3. Analysis of HIF-1 $\alpha$  protein stability. HCT116 cells were exposed to 100  $\mu$ M PGE<sub>2</sub> for 24 h (Lanes 1–3) or 100  $\mu$ M CoCl<sub>2</sub> for 4 h (Lanes 4–6). Cycloheximide was added to a final concentration of 100  $\mu$ M, and cells were harvested 0, 20, or 40 min later. The fraction of HIF-1 $\alpha$  remaining is indicated.

PGE<sub>2</sub> binds to the G protein-coupled receptors EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and EP<sub>4</sub>. The EP<sub>1</sub> receptor-selective agonist 17-pt-PGE<sub>2</sub> was as effective as PGE<sub>2</sub> in stimulating HIF-1 $\alpha$  protein expression in HCT116 cells (Fig. 2A). Furthermore, the EP<sub>1</sub> receptor-selective antagonist SC51322 inhibited PGE<sub>2</sub>-induced HIF-1 $\alpha$  expression in a dose-dependent manner (Fig. 2B). Neither 17-pt-PGE<sub>2</sub> nor SC51322 had any effect on HIF-1 $\beta$  expression. Thus, EP<sub>1</sub> receptor activation appears to be necessary and sufficient to induce HIF-1 $\alpha$  expression in HCT116 cells.

In HCT116 cells subjected to hypoxia or IGF-1 treatment, increased HIF-1 $\alpha$  protein levels result from a decreased rate of degradation and an increased rate of synthesis, respectively (16). To determine which of these mechanisms is involved in PGE<sub>2</sub>-induced HIF-1 $\alpha$  expression, cells were cultured for 24 h in the presence of PGE<sub>2</sub> or CoCl<sub>2</sub>, which inhibits HIF-1 $\alpha$  degradation, similar to the effect of hypoxia. The half-life of HIF-1 $\alpha$  protein was >40 min in CoCl<sub>2</sub>-treated cells but <20 min in PGE<sub>2</sub>-treated cells (Fig. 3). These results indicate that, unlike CoCl<sub>2</sub>, PGE<sub>2</sub> does not increase the half-life of HIF-1 $\alpha$  protein and therefore must stimulate HIF-1 $\alpha$  protein synthesis, similar to the effect of IGF-1.

To investigate the signal transduction pathways activated by PGE<sub>2</sub>, we first analyzed the phosphorylation status of ERK. Phosphorylation of ERK was induced with delayed kinetics in response to PGE<sub>2</sub> stimulation (Fig. 1A). PD98059 inhibits the activity of MEK, which is responsible for the activating phosphorylation of ERK. PD98059 blocked the induction of HIF-1 $\alpha$  protein and VEGF mRNA that was induced by PGE<sub>2</sub> (Fig. 4A). Wortmannin and rapamycin are inhibitors of PI3K and the downstream serine-threonine kinase FKBP rapamycin-associated protein (also known as mammalian target of rapamycin), respectively. Both Wortmannin and rapamycin partially inhibited PGE<sub>2</sub>-induced HIF-1 $\alpha$  protein and VEGF mRNA expression. Neither the inducers or inhibitors had any consistent effect on the expression of HIF-1 $\beta$  protein or HIF-1 $\alpha$  mRNA (Fig. 4A). In the presence of a

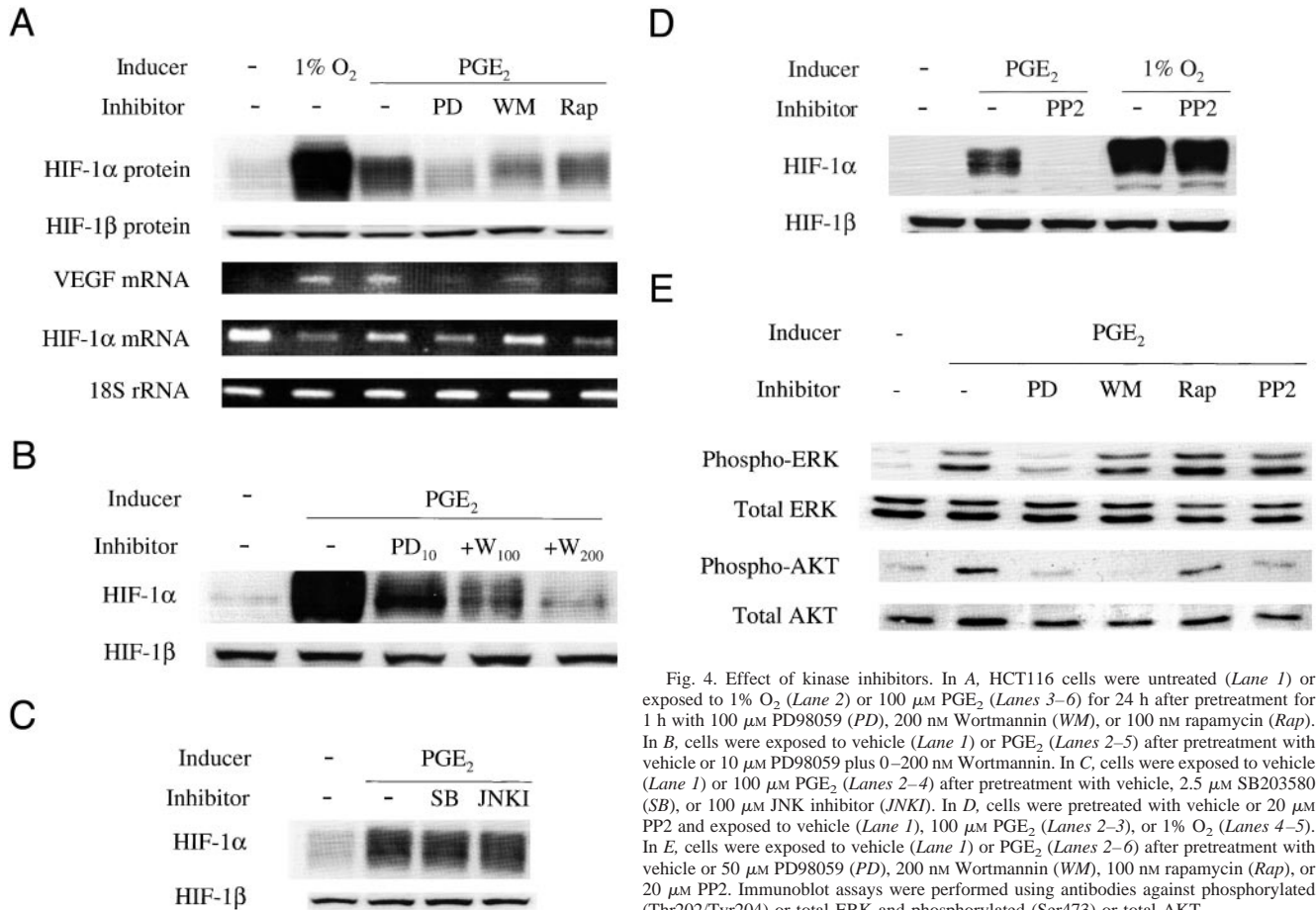


Fig. 4. Effect of kinase inhibitors. In A, HCT116 cells were untreated (Lane 1) or exposed to 1% O<sub>2</sub> (Lane 2) or 100  $\mu$ M PGE<sub>2</sub> (Lanes 3–6) for 24 h after pretreatment for 1 h with 100  $\mu$ M PD98059 (PD), 200 nM Wortmannin (WM), or 100 nM rapamycin (Rap). In B, cells were exposed to vehicle (Lane 1) or PGE<sub>2</sub> (Lanes 2–5) after pretreatment with vehicle or 10  $\mu$ M PD98059 plus 0–200 nM Wortmannin. In C, cells were exposed to vehicle (Lane 1) or 100  $\mu$ M PGE<sub>2</sub> (Lanes 2–4) after pretreatment with vehicle, 2.5  $\mu$ M SB203580 (SB), or 100  $\mu$ M JNK inhibitor (JNKI). In D, cells were pretreated with vehicle or 20  $\mu$ M PP2 and exposed to vehicle (Lane 1), 100  $\mu$ M PGE<sub>2</sub> (Lanes 2–3), or 1% O<sub>2</sub> (Lanes 4–5). In E, cells were exposed to vehicle (Lane 1) or PGE<sub>2</sub> (Lanes 2–6) after pretreatment with vehicle or 50  $\mu$ M PD98059 (PD), 200 nM Wortmannin (WM), 100 nM rapamycin (Rap), or 20  $\mu$ M PP2. Immunoblot assays were performed using antibodies against phosphorylated (Thr202/Tyr204) or total ERK and phosphorylated (Ser473) or total AKT.

submaximal concentration of PD98059, Wortmannin further decreased HIF-1 $\alpha$  protein expression in a dose-dependent manner (Fig. 4B). Inhibitors of two other MAP kinases, p38 and JNK, had no effect on PGE<sub>2</sub>-induced HIF-1 $\alpha$  protein expression (Fig. 4C).

PP2, an inhibitor of C-SRC tyrosine kinase activity, completely blocked HIF-1 $\alpha$  protein expression induced by PGE<sub>2</sub> but not by hypoxia (Fig. 4D). Although PD98059, Wortmannin, rapamycin, and PP2 all inhibited PGE<sub>2</sub>-induced HIF-1 $\alpha$  protein expression (Fig. 4A–D), only PD98059 blocked PGE<sub>2</sub>-induced ERK phosphorylation (Fig. 4E). These results indicate that neither C-SRC nor PI3K pathway activation was required for PGE<sub>2</sub>-induced ERK activation. PGE<sub>2</sub> induced phosphorylation of AKT that was blocked by pretreatment with Wortmannin (Fig. 4E). Pretreatment with PD98059 or PP2 partially inhibited PGE<sub>2</sub>-induced AKT phosphorylation, suggesting that PI3K-AKT signaling was dependent on both ERK and C-SRC activity.

The data presented above (Figs. 1, A and B and 4A) demonstrate a remarkably consistent correlation between HIF-1 $\alpha$  protein and VEGF mRNA expression in cells treated with various kinase inhibitors and/or PGE<sub>2</sub>. To further demonstrate that HIF-1 $\alpha$  is required for the induction of VEGF mRNA expression in response to PGE<sub>2</sub>, HCT116 cells were mock transfected or transfected with a small interfering RNA (siRNA<sub>HIF-1 $\alpha$</sub> ) that targets HIF-1 $\alpha$  mRNA for degradation. After transfection, the cells were serum starved and exposed to vehicle or PGE<sub>2</sub>. As expected, HIF-1 $\alpha$  mRNA levels were reduced in cells transfected with siRNA<sub>HIF-1 $\alpha$</sub>  (Fig. 5). VEGF mRNA levels were slightly reduced in siRNA<sub>HIF-1 $\alpha$</sub> -transfected cells treated with vehicle and dramatically reduced in PGE<sub>2</sub>-treated cells. Thus, inhibition of HIF-1 $\alpha$  expression is sufficient to block PGE<sub>2</sub>-induced VEGF mRNA expression.

## DISCUSSION

The present studies have delineated molecular mechanisms by which PGE<sub>2</sub> stimulates VEGF mRNA expression in human colon carcinoma cells, thus providing a link between COX2 activity and tumor angiogenesis. Although most experiments involved exposing cells to relatively high concentrations of PGE<sub>2</sub>, concentration-dependent effects were observed over the range of 1–100  $\mu$ M. Induction of VEGF mRNA expression is mediated by binding of PGE<sub>2</sub> to the EP<sub>1</sub> G protein-coupled receptor and activation of the MEK-ERK and PI3K-AKT pathways. We have demonstrated previously that transfection of HCT116 cells with an expression vector encoding constitutively active MEK results in HIF-1 $\alpha$  protein and VEGF mRNA expression (16). We demonstrate that, unlike hypoxia, PGE<sub>2</sub> treatment is not associated with an increase in the half-life of HIF-1 $\alpha$  protein. Our data suggest that, as in the case of IGF-1, PGE<sub>2</sub> increases the rate of HIF-1 $\alpha$  protein synthesis. The induction of HIF-1 $\alpha$  protein and

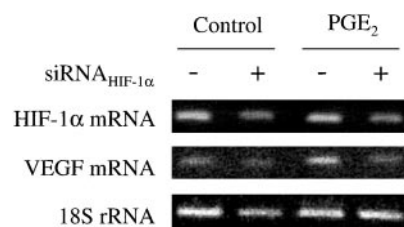


Fig. 5. Effect of HIF-1 $\alpha$  RNA interference on VEGF mRNA expression. HCT116 cells were exposed to Oligofectamine in the absence (–) or presence (+) of a small interfering RNA directed against HIF-1 $\alpha$  (siRNA<sub>HIF-1 $\alpha$</sub> ). A day after transfection, the cells were serum starved for 24 h and then exposed to vehicle (Control) or 100  $\mu$ M PGE<sub>2</sub> for 24 h.

VEGF mRNA expression in PGE<sub>2</sub>-treated cells is completely or partially blocked by inhibitors of MEK and PI3K, respectively, as was also observed in IGF-1-treated HCT116 cells (16). Inhibition of C-SRC tyrosine kinase activity also blocks the induction of HIF-1 $\alpha$  protein and VEGF mRNA expression in PGE<sub>2</sub>-treated cells without blocking ERK phosphorylation. Our data indicate that the signal transduction from PGE<sub>2</sub> receptor binding to HIF-1 $\alpha$  expression is complex, and additional studies are required to determine the mechanisms and consequences of ERK, PI3K, and C-SRC activation in PGE<sub>2</sub>-treated HCT116 cells.

Although HIF-1 $\alpha$  protein and VEGF mRNA expression are induced in HCT116 cells exposed to either IGF-1 or PGE<sub>2</sub>, there are several notable differences in the respective signal-transduction pathways: (a) in contrast to IGF-1, PGE<sub>2</sub> induces ERK phosphorylation and HIF-1 $\alpha$  expression with delayed kinetics, which suggests a requirement for gene expression; and (b) AKT phosphorylation is dependent on ERK activation in PGE<sub>2</sub>-treated HCT116 cells but independent of ERK activation in IGF-1-treated cells. PGE<sub>2</sub>-induced AKT phosphorylation is also dependent on C-SRC tyrosine kinase activity. Induction of HIF-1 $\alpha$  protein and VEGF mRNA expression in V-SRC-transfected rodent cells has been demonstrated previously (27).

While this study was in preparation, the induction of HIF-1 $\alpha$  expression by PGE<sub>2</sub> treatment of PC-3ML human prostate cancer cells was reported (28). HIF-1 $\alpha$  expression was induced by EP<sub>2</sub> and EP<sub>4</sub> but not EP<sub>3</sub> receptor-selective agonists, whereas the EP<sub>1</sub> receptor was not expressed. PD98059 inhibited PGE<sub>2</sub>-induced HIF-1 $\alpha$  expression. However, HIF-1 $\alpha$  expression was induced within 4 h in PGE<sub>2</sub>-treated PC-3ML cells (28). These results suggest that different signal transduction pathways are activated by PGE<sub>2</sub> in prostate and colon cancer cells, although MEK-ERK activation appears to play an essential role in both cases. The effect of PGE<sub>2</sub> in PC-3ML cells was attributed to stabilization of HIF-1 $\alpha$  protein, but no data were presented to support this conclusion. Additional studies are required to address this issue.

VEGF expression is induced in colon and other cancer cells as a result of hypoxia and multiple genetic alterations, including p53 and PTEN loss-of-function, RAS and SRC gain-of-function, and autocrine tyrosine kinase signaling pathways involving epidermal growth factor receptor, HER2<sup>neu</sup>, and IGF-1R (29–35). In each case, VEGF gene expression is activated by HIF-1 (16, 17, 27, 36–39). Based on the analysis of prostate cancer cells recently reported (28) and colon cancer cells presented in this study, this list can now be extended to include the increased VEGF expression resulting from COX2-generated PGE<sub>2</sub>. COX2 inhibitors, either alone or in combination with traditional cancer therapies such as radiation, have antiangiogenic effects (5, 40). As in the case of the tyrosine kinase-signaling pathways described above, the antiangiogenic effects of COX2 inhibitors appear to be attributable in part to their inhibition of HIF-1 $\alpha$  expression (28, 41). Efforts to identify small molecules that directly inhibit HIF-1 activity are under way (42), and such compounds may represent useful additions to the armamentarium of anticancer agents that target signal transduction pathways and angiogenesis.

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