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Prostaglandin $F_{2\alpha}$ stimulates motility and invasion in colorectal tumor cells

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Abstract

Increased expression of cyclooxygenase-2 (COX-2) and subsequent prostaglandin production is an important event in several human malignancies, including colorectal cancer. COX-2 mediated prostanoid synthesis has been shown to play a key role in tumor progression with prostaglandin E_2 (PGE_2) being shown to promote tumor growth, invasion and angiogenesis. The role of the other prostaglandins produced by COX-2 in tumors remains poorly understood. We have shown that colorectal tumor cells produce prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and provide evidence that $PGF_{2\alpha}$ may play an important role in colorectal tumorigenesis. Our data show that $PGF_{2\alpha}$ is secreted by both colorectal adenoma and carcinoma-derived cell lines at levels in excess of those detected for PGE_2. These cell lines were also found to express the $PGF_{2\alpha}$ receptor (FP) indicating potential autocrine effects of $PGF_{2\alpha}$. This finding is further supported by an *in vivo* immunohistochemical study of FP expression in resected colon tissue. These data show epithelial expression of FP in normal colorectal mucosa and also in colorectal adenomas and carcinomas. We compared the relative abilities of $PGF_{2\alpha}$ and PGE_2 to induce cell motility *in vitro* in colorectal tumor cell lines and show the first evidence of prostaglandin-induced cell motility in colorectal adenoma cell lines. $PGF_{2\alpha}$ induced cell motility with equivalent potency to PGE_2 in all the cell lines tested and was also shown to increase the invasion of carcinoma-derived cells into reconstituted basement membrane. These data show that $PGF_{2\alpha}$ may play an important role in the malignant progression of colorectal tumors.

Keywords

colon; cancer; adenoma; prostaglandin; motility; invasion

Colorectal cancer remains a major cause of cancer deaths globally. The inducible form of cyclooxygenase (COX-2), the key enzyme in prostanoid biosynthesis, is overexpressed in over 80% of colorectal cancers.1 Furthermore, COX-2 is frequently expressed during the premalignant adenoma stages of colorectal tumorigenesis and shows a size dependent increase in expression indicating a role in tumor progression.2

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The importance of COX-2 expression in colorectal tumor progression has been highlighted by studies both *in vitro* and *in vivo*, and its effects are mediated through its prostanoid products.3 COX-2 catalyses the formation of prostaglandin H_2 (PGH_2) from arachidonic acid and PGH_2 is subsequently converted to several structurally related primary prostanoids, namely prostaglandin E_2 (PGE_2), PGD_2, PGI_2, Thromboxane A_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by the action of specific synthases. Increased levels of PGE_2 have been observed in colorectal cancers when compared with histologically normal tissue.4-6 Subsequent studies using *in vitro* models have shown that PGE_2 is able to promote tumor cell growth,7,8 modulate apoptosis9 and increase cell motility.8 COX-2 expression and subsequent PGE_2 production has also been shown to enhance the production of angiogenic factors.10-12

Studies of prostaglandin production in colorectal tumors have also identified the production of other primary prostanoids including $PGF_{2\alpha}$.4-6 In 1 study on intestinal tissue from familial adenomatous polyposis (FAP) patients, the detected levels of $PGF_{2\alpha}$ were 30-fold higher than for PGE_2.13

Although widely studied in other tissues, the potential role of $PGF_{2\alpha}$ in colorectal cancer remains to be elucidated. The most extensively studied area of $PGF_{2\alpha}$ biology is its role in the regression of the corpus luteum during pregnancy.14 Interestingly, $PGF_{2\alpha}$ stimulates the proliferation of Swiss mouse 3T3 cells with greater efficacy than PGE_2.15,16

$PGF_{2\alpha}$ is thought to largely act through the FP G-protein-coupled receptor, although the expression of this receptor has not been determined in colorectal cancer. Because of the importance of COX-2 overexpression, the aim of the study presented here was to investigate the potential role of $PGF_{2\alpha}$ in colorectal tumorigenesis. To this end, we determined the expression of the FP receptor in normal and neoplastic colorectal tissue, and a panel of adenoma and carcinoma-derived cell lines. The production of $PGF_{2\alpha}$ was examined in parallel with that of PGE_2 in the cell lines. Finally, we assessed the ability of both $PGF_{2\alpha}$ and PGE_2 to promote cell motility and invasion which represent important hallmarks of malignant progression in these tumors.

Material and methods

Cell lines and culture conditions

AA/C1 is a clonogenic, adenoma cell line derived from a 3 to 4 cm polyp from the descending colon of a familial adenomatous polyposis (FAP) patient17 and is cultured in conditioned medium as described by Williams *et al.*18 RG/C2 is a clonogenic cell line derived from a sporadic tubular adenoma of the sigmoid colon of 1–2 cm in diameter and is cultured in DMEM supplemented with 20% (vol/vol) FBS (Autogen Bioclear, Calne, UK). 19 Both of these adenoma-derived cell lines are anchorage-dependent and are nontumorigenic in athymic nude mice. HCA7 was established from a moderately well differentiated mucinous carcinoma of the colon20 and was a kind gift from Dr. Sue Kirkland (London, UK). HCA7-col29 was sub-cloned from the parental line21 and will be hereafter referred to as HCA7. SW480 was derived from a sporadic colonic adenocarcinoma.22 The carcinoma lines were cultured in DMEM supplemented with 10% (vol/vol) FBS, and all cell lines were cultured as adherent cells in 25 cm^2 tissue culture flasks.

For growth response assays, cells were seeded at 10^6 per 25 cm^2 flask and allowed to recover for 48 hr. The medium was then changed to DME-F12 (Invitrogen, Paisley, UK), supplemented with 2% FBS, containing the appropriate amount of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Sigma, Poole, UK) or vehicle control. A range of prostaglandin concentrations from 0.1 to 10 μM was tested and compared with vehicle and untreated control. Treatments were made from prepared stocks so that a 1:1,000 dilution of 95% ethanol was added to each

treated flask. Following a 72 hr treatment period, attached cell yield and the proportion of apoptotic cells that had detached from the culture substrate were quantified using a haemocytometer. The level of apoptosis in cultured colonic cells can be assessed by measuring the proportion of cells that detach from the flask and float in the medium.23,24 Apoptosis was confirmed in these 'floating' cells by morphology (following acridine orange staining).

Prostaglandin production assay

$PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2) enzyme immunoassay kits (Cayman Chemical Company, Ann Arbor, MI) were used to assay the release of $PGF_{2\alpha}$ and PGE_2 into the growth medium by the cells. The lower limit of detection of the assays was 5.5 pg/ml for $PGF_{2\alpha}$ and 36 pg/ml for PGE_2, and the upper limit of detection was 64 pg/ml and 250 pg/ml for $PGF_{2\alpha}$ and PGE_2, respectively. Cells were grown to ~70% confluency and the standard culture medium replaced with serum-free DMEM-F12 (Invitrogen, Paisley, UK) for a period of 72 hr whereupon the medium was removed, centrifuged and decanted, snap-frozen in liquid nitrogen and stored at −70°C prior to analysis. These samples were diluted appropriately so that readings fell within the detection limits of the assay. $PGF_{2\alpha}$ and PGE_2 production was normalized according to the number of adherent cells present in the particular culture at the time of sampling. The results are expressed as picograms of prostaglandin/10^6 cells and represent the average of 2 independent experiments performed in duplicate.

In vitro motility and invasion assays

Cell motility assays were carried out using a transwell filter motility assay as previously described.25 For quantitative analysis of cell motility 8 μm pore size insert filters (Becton Dickinson, Oxford, UK) were coated with 10 μg ml^{-1} Vitrogen Type I collagen (Cohesion, Palo Alto, CA). For invasion assays, 8 μm transwell filters precoated with Matrigel (Becton Dickinson, Oxford, UK) were used. For both types of analysis 1×10^5 cells were seeded per well in calcium-free DMEM containing 0.1% FBS (CF-DMEM) and allowed to adhere for 4 hr.

Initial experiments using a range of doses of prostaglandins from 0.1 to 10 μM [$PGF_{2\alpha}$ or PGE_2 (Sigma, Poole, UK) diluted 1:1,000 from a 1 mM stock made up in 95% ethanol, or vehicle (1:1,000 dilution of 95% ethanol)] were performed, 1 μM was selected as it was found to stimulate significant motility.

Following a 24 hr incubation, cells were removed from the upper filter surface with a cotton swab. The filters were fixed and stained with haematoxylin. Cells that had moved to the lower filter surface were counted in 10 fields at 20× magnification. Three independent experiments were carried out in triplicate, and the data are expressed as the mean ± SEM. Statistical analysis of this data was performed using the Student's *t* test. Differences were considered significance when the *p* value was <0.05.

Western blot analysis

Samples of 2×10^6 cells were prepared for Western blotting as described previously.26 The FP receptor was detected using a rabbit polyclonal antibody (1:1,000 dilution; Cayman Chemical Cayman, Ann Arbor, MI) and the Lumiglo ECL detection system (Amersham Biosciences, Little Chalfont, UK). Ishikawa endometrial carcinoma cells stably overexpressing the FP receptor were included as a positive control.27 Anti α-tubulin (diluted 1:1,000; Sigma, Poole, UK) was used as a loading control.

Immunohistochemistry

Immunohistochemical analysis of FP receptor expression was carried out as previously described.27 Following approval from the local research ethics committee, anonymised paraffin-embedded, formalin-fixed archival material was retrieved from the files of the Department of Histopathology, Bristol Royal Infirmary for use in this study. A total of 15 colorectal adenomas were examined. These included 5 small (≤5 mm) tumors, 5 medium-sized (5–10 mm) tumors and 5 large (≥10 mm) tumors. We also examined 10 invasive adenocarcinomas taken from colorectal resection specimens. Five samples of histologically normal mucosa were also included in the study.

The FP receptor was detected using a FP-specific affinity purified rabbit polyclonal antibody (diluted 1:500; Cayman, Ann Arbor, MI) and a biotinylated swine anti-rabbit secondary antibody (diluted 1:500; Dako Cytomation, Ely, UK). The specificity of the antibody was confirmed by prior overnight incubation at 4°C with blocking peptide (diluted 1:50; Cayman, Ann Arbor, MI). This preblocking was used as a negative control as shown in Figure 2*b*. A standard avidin-biotin immunoperoxidase technique (Dako Cytomation, Ely, UK) was employed and the immunoreaction visualized by means of the diamino benzidine reaction with haemotoxylin counterstaining. Antigen retrieval was performed by treating sections for 5 min in a pressure cooker in 0.1% citrate buffer (pH 6.0). Sections were examined for FP immunoreactivity.

Results

Prostaglandin $F_{2\alpha}$ is produced by colorectal adenoma and carcinoma cells

Samples of media were taken following 72 hr incubation of the adenoma-derived cell lines AA/C1 and RG/C2, and from the carcinoma-derived cell lines HCA7 and SW480. For this culture period, serum-free media was used and these samples were assayed for both PGE_2 and $PGF_{2\alpha}$ content using enzyme immunoassay. Prostaglandin concentrations were calculated in pg per million cells. Figure 1 shows the mean value obtained, and Table I the range of results from 2 independent experiments performed in duplicate.

Although relatively low amounts of PGE_2 were found in the adenoma-derived cell lines AA/C1 and RG/C2 (6.69 and 7.49 pg/million cells, respectively), both produced $PGF_{2\alpha}$ at a level comparable with that detected in SW480 carcinoma cells. The HCA7 carcinoma line produces very high levels of PGE_2 due to the relatively high levels of cyclooxygenase-2 (COX-2) expression in these cells caused by stabilization of the *COX-2* mRNA.28 It is interesting to note that HCA7 also produced higher levels of $PGF_{2\alpha}$ than PGE_2.

The FP prostaglandin receptor is expressed in colorectal tumors and in tumor-derived cell lines

The major receptor for $PGF_{2\alpha}$ has been designated FP and has been shown to be a G_q-linked G-protein coupled receptor which mediates its effects through modulating intracellular calcium and inositol phosphate.29 To our knowledge, no previous studies have reported the expression of the FP receptor in normal human colon tissue, colorectal tumors or in colorectal adenoma and carcinoma cell lines. *In vivo*, prostaglandins can be produced by both the epithelial and stromal components of the tumor and may act in an autocrine or paracrine fashion on neighboring cell types, eliciting a range of biological effects. To determine the possible target tissues of $PGF_{2\alpha}$ in colorectal tumors we used immunohistochemistry on resected human tissues to determine the expression pattern of the FP receptor *in vivo* (Fig. 2). The data presented are representative of 5 samples of normal colonic epithelium resected from patients with diverticulitis; 15 samples of benign colonic

adenoma and 10 samples of colorectal adenocarcinoma. The colorectal adenomas were divided according to size (small = <5 mm; medium = 5–10 mm; large = >10 mm).

Strong positive immunoreactivity to the FP receptor was seen in the epithelial component of normal colonic mucosa (100% (*n* = 5) Fig. 2*a* panel A) compared with the negative control (Fig. 2*a* panel B), yet no staining was detected in stromal cells. All of the colorectal adenomas (15/15) displayed positive epithelial immunoreactivity for FP but no expression was observed in any stromal tissues (Fig. 2*a* panels C–F).

The results in colorectal carcinomas were more varied. Carcinomas 7/10 displayed positive immunoreactivity for FP in the epithelium with no staining detected in stromal constituents (Fig. 2*a* panels G–I). Positive immunoreactivity for the FP receptor was detected in all of the normal and adenoma samples, and the majority of the carcinomas, both the intensity and distribution of the FP staining were observed to be similar when comparing normal with tumor tissue.

Having shown that the FP receptor was expressed in normal and tumor tissue *in vivo* we then studied the expression of the FP receptor in adenoma and carcinoma-derived cell lines by Western blotting. Representative results of 3 repeated experiments are shown in Figure 2*b*. Ishikawa endometrial carcinoma cells stably overexpressing the FP receptor were included as a positive control.27 Both of the adenoma cell lines and all 3 carcinoma cell lines show expression of the FP receptor. A doublet of bands was seen for each cell line and it is not possible, using the reagents currently available, to determine whether these represent 2 distinct isoforms of the receptor or some form of post-translational modification.27 However, these data do show the expression of the FP receptor in normal and neoplastic colorectal epithelium, suggesting a possible autocrine role for $PGF_{2\alpha}$ secreted by the cells.

Prostaglandins $F_{2\alpha}$ and E_2 stimulate cell motility in colorectal adenoma-derived cell lines

Previous studies have shown that PGE_2 confers broad ranging tumor-promoting effects in colorectal cancer yet nothing is currently known about the role of $PGF_{2\alpha}$ in this disease. Having shown that colorectal tumor cells produce $PGF_{2\alpha}$ and that the FP receptor is expressed by colorectal epithelial cells *in vitro* and *in vivo*, we investigated the potential role of $PGF_{2\alpha}$ in colorectal tumorigenesis using *in vitro* models.

Previous studies have reported that exogenous addition of $PGF_{2\alpha}$ stimulated cell growth in the colorectal carcinoma cell line SW1116,7 but produced no significant effects on growth in HCT8 and HT29.30 Studies in this laboratory have shown that cell lines derived from colorectal adenomas31 and carcinomas32 are growth stimulated by exogenous addition of PGE_2. We conducted $PGF_{2\alpha}$ growth response experiments on the cell lines studied above (adenomas: AA/C1, RG/C2; carcinomas: SW480, HCA7) with a range of concentrations from 0.1 to 10 μM. No significant change in cell growth or apoptosis was detected in any of the cell lines studied over a 72 hr period (data not shown).

Previous work has shown that PGE_2 has multiple effects on colorectal carcinoma cells, including increasing cell motility.8,33 There are no previous reports of $PGF_{2\alpha}$ inducing motility in tumor cells and, importantly, no studies of prostaglandin-induced motility in colorectal adenomas. We compared the ability of exogenous $PGF_{2\alpha}$ and PGE_2 to influence the motility of colorectal adenoma (AA/C1, RG/C2) and carcinoma (SW480, HCA7) cells using a Boyden-chamber transwell-filter cell motility assay, compared to solvent control. Initial experiments were performed using a range of doses of prostaglandins (0.1–10 μM) and 1 μM was selected as it was found to stimulate significant motility. Cells were treated and incubated for 24 hr to allow movement through the collagen-coated filters. Figure 3*a* shows the average results of 3 independent experiments performed in triplicate. As would be

expected, the adenoma derived cell lines AA/C1 and RG/C2, which represent earlier stages of colorectal tumor progression, were less motile than their carcinoma counterparts, which display a ~2-fold higher rate of motility (Fig. 3*a*).

Both $PGF_{2\alpha}$ and PGE_2 significantly increased cell motility in adenoma- (AA/C1 and RG/C2, $p = <0.001$) and carcinoma-derived (HCA7 and SW480, $p = <0.001$) cell lines. These are the first data showing the ability of prostaglandins to increase motility in adenoma cells which represent the earlier, nonmalignant stages of colorectal tumor development.

Prostaglandin $F_{2\alpha}$ stimulates invasion in carcinoma cells

The process by which tumor cells break out from their site of origin and metastasise to distant sites requires, in addition to motility, an ability to invade through the basement membrane and underlying mesenchymal cells. This process can be modeled *in vitro* by coating transwell filters (as used for the motility assay above) with reconstituted basement membrane, in this instance Matrigel™. PGE_2 has been shown previously to stimulate invasion by colorectal cancer cells in this type of assay.34 The adenoma-derived cell lines have been shown previously to be noninvasive [Ref. 35 and Qualtrough and Paraskeva, unpublished observations] in keeping with their premalignant nature. Adoption of an invasive phenotype is a key distinction between benign and malignant colorectal tumors.

The data presented in Figure 3*b* clearly shows that 1 μM $PGF_{2\alpha}$ significantly stimulates invasion of HCA7 and SW480 colon carcinoma cells into a reconstituted basement membrane (HCA7, $p = <0.05$; SW480, $p = <0.01$). The figures show a 1.8-fold stimulation of invasion in HCA7 and a 1.5-fold increase in SW480 with $PGF_{2\alpha}$ compared with control.

Discussion

The overexpression of COX-2 is an important event in colorectal cancer. It has also become clear that it is the prostanoid products of COX-2 activity that mediate major effects on tumor cell behavior by promoting growth, survival, invasive behavior and inducing angiogenesis.36 Much of the focus of prostanoid research in the area of colorectal cancer has fallen on PGE_2, widely accepted to be the major prostanoid product of COX-2 in these tumors.31 COX-2 overexpression also leads to the production of other primary prostanoids such as PGD_2, PGI_2 and $PGF_{2\alpha}$, although the role of these in colorectal tumorigenesis remains poorly understood.36 Several researchers have detected $PGF_{2\alpha}$ production in colorectal tumor tissue, and in 1 study the levels detected were found to be in excess of those for PGE_2.13 The purpose of the study presented here was to determine the potential role of $PGF_{2\alpha}$ in colorectal tumorigenesis.

The data presented here clearly shows readily detectable levels of $PGF_{2\alpha}$ produced by cell lines derived from both colorectal adenomas and carcinomas. Considering the known importance of PGE_2 in colorectal tumorigenesis, it is of interest that the levels of $PGF_{2\alpha}$ found were in excess of those of PGE_2, suggesting that $PGF_{2\alpha}$ may be of biological importance in these tumors.

The primary prostanoids have been shown to function through specific prostanoid receptors which, in the case of $PGF_{2\alpha}$, has been designated FP.27,37 Although there are no published studies on the expression of FP in the human lower gastrointestinal tract, 1 previous study showed FP expression in the epithelium of the stomach.38 We have now shown for the first time that the FP receptor is expressed in the normal colonic epithelium, and also in the epithelial component of colorectal adenomas and carcinomas. Interestingly, no significant discernible difference was observed in the intensity of FP immunoreactivity between normal and tumor samples. However, the greatly altered signaling context of the neoplastic tissue

could elicit different cellular responses to $PGF_{2\alpha}$. Consistent with these *in vivo* studies, we showed the expression of the FP receptor in cell lines derived from both colorectal adenomas and carcinomas. The presence of the $PGF_{2\alpha}$ ligand and the expression of the FP receptor in colorectal tumor epithelial cells imply a functionality to this pathway. To determine the potential role of $PGF_{2\alpha}$ in colorectal tumorigenesis we used an *in vitro* model system. We found no significant effects on cell growth or apoptosis in response to $PGF_{2\alpha}$ in either the adenoma or carcinoma cell lines tested. This finding is in relation with another published study on the HCT8 and HT29 cell lines,30 where no growth stimulation was observed. Similarly, Qiao *et al.* also showed no proliferative response in HT29 cells to $PGF_{2\alpha}$, whereas SW116 were growth stimulated, suggesting that the cellular response to $PGF_{2\alpha}$ may be tumor specific.7

The acquisition of a motile phenotype is a hallmark of colorectal tumor progression. Previous studies have shown that PGE_2 can stimulate motility in the colorectal carcinoma cell line LS174T.8 No previous study has examined the ability of prostaglandins to promote motility in colorectal adenoma cells and there are no reports of $PGF_{2\alpha}$ affecting cell motility in colonic epithelial cells. We compared the effects of $PGF_{2\alpha}$ and PGE_2 on cell motility in both adenoma and carcinoma-derived cell lines. We report the first evidence of prostaglandin-induced cell motility in adenoma cells which responded to both $PGF_{2\alpha}$ and PGE_2. Our data also show that $PGF_{2\alpha}$ significantly stimulates cell motility in colorectal carcinoma cell lines, as well as adenomas, and is comparable with PGE_2 in its ability to do so. These data show an important biological effect of $PGF_{2\alpha}$ in colonic tumor cells and also suggest that prostaglandins may contribute to the progression of the adenoma to carcinoma sequence by increasing cell motility.

The process of tumor invasion (and therefore malignancy) requires cell motility but also alterations in cell adhesion and the secretion of enzymes to degrade basement membrane and matrix components. Pai *et al.* have reported that PGE_2 potentiates invasiveness in the colorectal carcinoma cell lines SW480 and LoVo although the effects of $PGF_{2\alpha}$ remain unreported.34 We have now shown that $PGF_{2\alpha}$ can also increase the invasiveness of the SW480 and HCA7 carcinoma cell lines *in vitro*, with the SW480 data being comparable with that previously published for PGE_2.34 These increases were statistically significant in both of the cell lines and demonstrate a potentially important and novel tumorpromoting effect of $PGF_{2\alpha}$ in colorectal cancer. Because of the complexity of the invasion process, it is possible that $PGF_{2\alpha}$ may also stimulate the production of matrix remodeling enzymes and alter cell adhesion complexes, in addition to stimulating cell motility. PGE_2 has been shown to stimulate colon cancer cell invasion through transactivation of the epidermal growth factor receptor (EGFR).34 We have not seen EGFR activation by $PGF_{2\alpha}$ in our system (data not shown), suggesting alternate mechanisms of action for this prostaglandin which represents an interesting area for potential future study.

In conclusion, the data presented here show that $PGF_{2\alpha}$ may be an important product of COX-2 overexpression in colorectal tumors, by promoting tumor progression and potentially metastasis. The use of high doses of NSAIDs in colorectal cancer prevention and therapy has recently come under close scrutiny due to the adverse cardiovascular effects associated with long term use of Rofecoxib and the subsequent withdrawal of this drug from clinical use.39 Although this does not preclude the use of this type of drug in an adjuvant setting, it does highlight the need for more targeted approaches.40,41 To facilitate this, it is of key importance that we increase our understanding of the pleiotropic effects of the various prostanoid products of the COX-2 enzyme. The data presented here show that $PGF_{2\alpha}$, as well as the widely-studied PGE_2 needs to be considered in our understanding of colorectal cancer.

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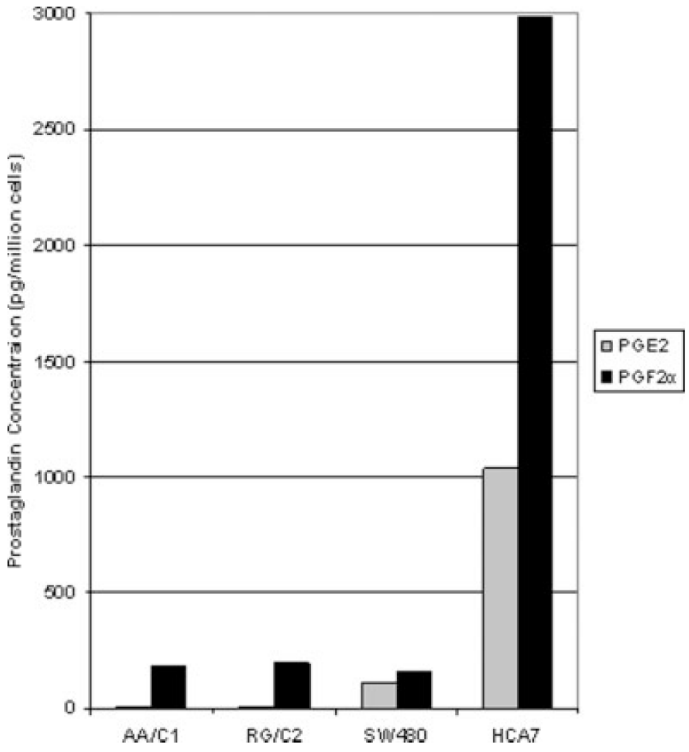

Figure 1.

Colorectal tumor cells produce PGF2α. Graphical representation of $PGF_{2\alpha}$ and PGE_2 produced by colorectal adenoma and carcinoma cell lines. Enzyme immunoassays for $PGF_{2\alpha}$ and PGE_2 were performed on serum-free media from each cell line following 72 hr incubation. AA/C1 and RG/C2 are adenoma-derived cell lines, whereas SW480 and HCA7 are carcinoma-derived. Prostaglandin production was calculated as picogrammes per million cells and these data represent the mean of 2 experiments performed in duplicate.

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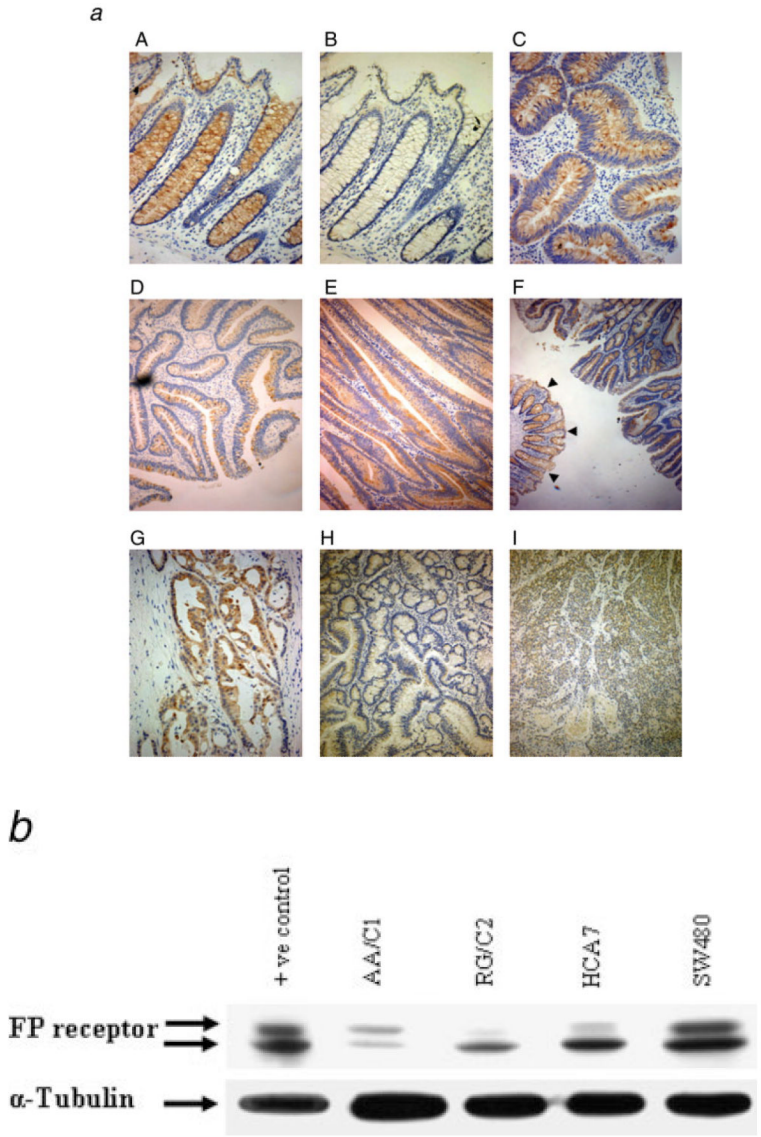

FIGURE 2 – CONTINUED

Figure 2.

The FP receptor is expressed in resected colorectal adenomas, carcinomas and tumor derived cell lines. (*a*) Sections of resected human tissue were immunohistochemically stained with FP-specific antibody and digitally photographed on a light microscope. This figure shows representative views selected from data for: 15 colorectal adenomas [5 small (≤5 mm), 5 medium (5–10 mm) and 5 large (≥10 mm) tumors]; 10 invasive adenocarcinomas (3 well differentiated, 3 moderately differentiated and 4 poorly differentiated); and 5 samples of normal mucosa taken from diverticulitis patients. A: Normal (×200 magnification); B: normal—corresponding negative control (preabsorbed over-night with FP blocking peptide) (×200); C: small adenoma (×200); D: medium adenoma (×100); E: large adenoma (×100); F: large adenoma, low magnification showing normal (arrows) and adenoma from the same surgical resection (×25); G: moderately differentiated carcinoma (×200); H: well differentiated carcinoma (×100); I: poorly differentiated carcinoma (×100). (*b*) Expression of the FP receptor in colorectal tumor cell lines. Western blots were carried out on samples

from the colorectal tumor cell lines using an FP-specific antiserum. The FP receptor runs as a doublet in the FP-overexpressing endometrial carcinoma cell line used as a positive control, with the lower band at ~64 kDa.25 The presence of the FP receptor was detected in all of the cell lines examined. The blot was reprobed with α-tubulin to control for equal loading of samples. Expression analysis was carried out in 3 repeated experiments and the results shown are representative.

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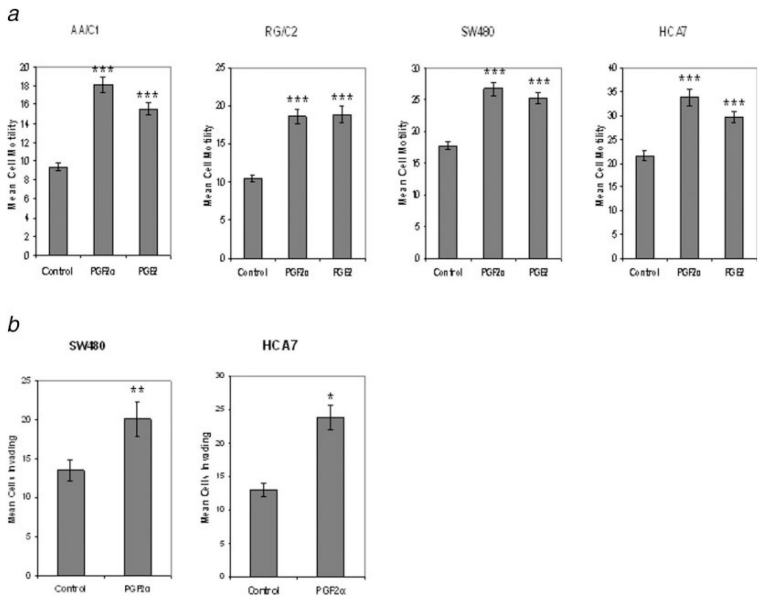

Figure 3.

PGF2α-induced motility and invasion in colorectal tumor cells. (*a*) The effects of 1 μM $PGF_{2\alpha}$ or PGE_2 on the motility of colorectal adenoma- (AA/C1 & RG/C2) and carcinoma-derived (SW480 & HCA7) cell lines as measured by collagen-coated transwell filter assay after 24 hr of treatment, compared to solvent control. The data are expressed as the total cells observed to have migrated from 10 fields of view (±SEM) and represent 3 separate experiments performed in triplicate. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ prostaglandin *vs.* solvent control. (*b*) The effects of 1 μM $PGF_{2\alpha}$ on the invasion of colorectal carcinoma cell lines into Matrigel™ after 24 hr of treatment, compared to solvent control. The data are expressed as the total cells observed to have invaded from 10 fields of view (±SEM) and represent 3 separate experiments performed in triplicate. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ prostaglandin *vs.* control.

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TABLE I

COLORECTAL TUMOR CELLS PRODUCE $PGF_{2\alpha}$

	Cell line			
	AA/C1	**RG/C2**	**SW480**	**HCA7**
Prostaglandin				
PGE_2 (pg/million cells)	5.20–8.18	5.12–9.86	86.21–123.69	910.39–1157.61
$PGF_{2\alpha}$ (pg/million cells)	157.14–203.10	175.52–217.96	139.35–178.69	2777.26–3193.16

The table gives a numerical representation of the data presented in Figure 1. These data represent the range of values obtained from 2 experiments performed in duplicate. AA/C1 and RG/C2 are adenoma-derived cell lines, whereas SW480 and HCA7 are carcinoma-derived.