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Expression of COX-2 and PGE synthase and synthesis of PGE_2 in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors

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Summary This study was designed to investigate the possible role of cyclo-oxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) in endometrial adenocarcinoma. COX-2 RNA expression was confirmed in various grades of adenocarcinoma by ribonuclease protection assay. COX-2 and microsomal glutathione-dependent prostaglandin E synthase (mPGES) expression and PGE_2 synthesis were localised to the neoplastic epithelial cells and endothelial cells. In order to establish whether PGE_2 has an autocrine/paracrine effect in adenocarcinomas, we investigated the expression of 2 subtypes of PGE_2 receptors, namely EP2 and EP4, by real time quantitative PCR. Expression of EP2 and EP4 receptors was detected in adenocarcinomas from all grades of differentiation and was significantly higher than that detected in normal secretory phase endometrium ($P < 0.01$). The fold induction of expression in adenocarcinoma compared with normal secretory phase endometrium was 28.0 ± 7.4 and 52.5 ± 10.1 for EP2 and EP4 receptors respectively. Immunohistochemistry localised the site of expression of EP4 receptor in neoplastic epithelial cells and in the endothelium of carcinomas of all grades of differentiation. Finally, the functionality of the EP2/EP4 receptors was assessed by investigating cAMP generation following in vitro culture of adenocarcinoma tissue in the presence or absence of 300 nM PGE_2. cAMP production in response to PGE_2 was significantly higher in carcinoma tissue than that detected in normal secretory phase endometrium (3.42 ± 0.46 vs 1.15 ± 0.05 respectively; $P < 0.001$). In conclusion, these data suggest that PGE_2 may regulate neoplastic cell function in an autocrine/paracrine manner via the EP2/EP4 receptors. © 2001 Cancer Research Campaign http://www.bjcancer.com

Keywords: COX-2; prostaglandin; receptors; uterus; adenocarcinoma

Cyclo-oxygenase (COX) enzymes, also called prostaglandin endoperoxide synthase, catalyse the rate-limiting step in the conversion of arachidonic acid to prostaglandin H_2 (PGH_2). In turn PGH_2 serves as a substrate for specific prostaglandin synthase enzymes that synthesise the natural prostaglandins. These are named according to the prostaglandin they produce such that prostaglandin D_2 is synthesised by prostaglandin-D-synthase, prostaglandin E_2 (PGE_2) by prostaglandin-E-synthase (PGES) and prostaglandin F_2, by prostaglandin-F-synthase. To-date, there are 2 identified isoforms of the COX enzyme. COX-1 and COX-2 (DeWitt 1991). COX-1 is constitutively expressed in many tissues and cell types and generates prostaglandins for normal physiological function (Herschman, 1996). By contrast, the expression of COX-2 is rapidly induced following stimulation of quiescent cells by growth factors, oncogenes, carcinogens and tumour-promoting phorbol esters (Herschman, 1996; Subbaramaiah et al, 1996). In addition, 2 isoforms of PGES have been isolated: a microsomal glutathione-dependent inducible PGES (mPGES) and a constitutive cytosolic glutathione-dependent PGES (Jakobsson et al, 1999; Tanioka et al, 2000). In vitro studies support the idea that COX-2 and possibly PGE_2 are involved in neoplastic transformation of epithelial cells and subsequently carcinogenesis. Over-expression of COX-2 and PGE_2 synthesis in rat intestinal epithelial cells increases their proliferation rate, resistance to apoptosts, and their invasiveness by suppressing the transcription of target genes that may be involved in cellular growth/transformation and adhesion (Tsujii and DuBois, 1995). In addition, it has been proposed recently that COX-2 and PGE_2 promote cancer development and invasiveness by mediating the transcription of angiogenic factors that induce both migration of endothelial cells and their arrangement into tubular structures (Tsujii et al, 1998; Jones et al, 1999b).

PGE_2 mediates its effect on target cells through interaction with different isoforms of 7 transmembrane G protein-coupled receptors (GPCR) which belong to the rhodopsin family of serpentine receptors. Four main PGE_2 receptor subtypes have been identified (EP1, EP2, EP3 and EP4) which utilise alternate and in some cases opposing intracellular signalling pathways (Coleman et al, 1994). This diversity of receptors with opposing action may confer a homeostatic control on the action of PGE_2 that is released in high concentrations close to its site of synthesis (Ashby, 1998). To-date, the role of the different PGE_2 receptors, their divergent intracellular signalling pathways, as well as their respective target genes involved in mediating the effects of PGE_2 on normal or neoplastically transformed endometrial epithelial cells remain to be elucidated.

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Epithelial cells of the human endometrium are highly vulnerable to neoplastic transformation. In the Western world, endometrial carcinoma is the most common gynaecologic malignancy. Endometrial cancer can arise from several cell types but the glandular epithelium is the most common progenitor (adenocarcinomas account for 80–90% of uterine tumours). Endometrial cancer is predominantly a post-menopausal disease where incidence is uncommon below the age of 40 and peaks by about 70 years of age. The incidence of endometrial cancer has been increasing steadily in the Western world during the last 50 years and this has been attributed largely to increased life expectancy and improved detection methods (Gordon and Ireland, 1994; Mant and Vessey, 1994). This study was designed to investigate whether COX-2 and mPGES expression and PGE_2 synthesis are up-regulated in adenocarcinoma of the human uterus. In addition, a possible autocrine/paracrine role for PGE_2 in endometrial carcinogenesis was assessed by investigating (a) the expression of EP2/EP4 receptors in carcinoma tissue and (b) the effect of exogenous treatment of carcinoma tissue with PGE_2 on cAMP turnover.

MATERIALS AND METHODS

Tissue collection and processing

Endometrial adenocarcinoma tissue was collected from women undergoing hysterectomy and who had been pre-diagnosed to have adenocarcinoma of the uterus. All women with endometrial adenocarcinoma were post-menopausal. To provide control tissue, normal secretory phase (days 18–25 of the menstrual cycle) endometrial tissue was collected with a pipelle suction curette (Pipelle; Laboratoire CCD. Paris, France) from fertile women with regular menstrual cycles, undergoing gynecological procedures for benign conditions. Biopsies were dated from the patient's last menstrual period (LMP) and histological dating was consistent with date of LMP. Subjects had not been exposed to exogenous hormones for at least 6 months prior to inclusion in the study. This phase of the menstrual cycle was chosen for comparison with endometrial adenocarcinoma tissue as minimal proliferative activity of the endometrium is detected during the secretory phase (Ferenczy et al, 1979). This would be comparable to the absence of proliferative activity predicted in healthy post-menopausal endometrium. Shortly after hysterectomy or pipelle suction, the tissue was either snap frozen in dry ice and stored at –70˚C (for RNA extraction), fixed in neutral-buffered formalin and wax-embedded (for immunohistochemical analyses) or placed in RPM1 1640 (containing 2 mmol l^{-1} L-glutamine, 100 U penicillin and 100 µg ml^{-1} streptomycin) and transported to the laboratory for in vitro culture. In addition, archival tissue blocks of healthy post-menopausal uterus were obtained from The Department of Pathology (The University of Edinburgh Medical School) and utilised for immunohistochemical analyses. Written informed consent was obtained prior to tissue collection and ethical approval was received from the Lothian Research Ethics Committee. The data in this study were analysed by ANOVA using StatView 5.0.

Ribonuclease protection assay

RNA was extracted from endometrial adenocarcinoma tissue ($n = 3$ of each of well, moderately or poorly differentiated endometrial adenocarcinoma) and normal secretory phase endometrium ($n = 4$) using Tri-Reagent as recommended by the manufacturer (Sigma, Dorset, UK). A homologous 381 bp COX-2 cDNA probe was generated by PCR from a clone containing the human COX-2 cDNA (the clone was a gift from Dr S Prescott, University of Utah) and primers at base pair position 950–971 (COX2A: 5′-CAAGCAGGCTAATACTGATAGG-3′) and 1310–1331 (COX2B: 5′-ATCTGCCTGCTCTGGTCAATGG-3′). The amplified PCR product was subcloned into pCRII and its identity and orientation confirmed following sequencing using the Applied Biosystems 373A DNA sequencer and the ABI prism DNA sequencing kit (Applied Biosystems, Cheshire, UK).

For the RPA, an antisense cRNA probe was prepared from HindIII linearised pCRII plasmid containing the 381 bp cDNA fragment of the human COX-2. The RPA was conducted using the Ambion RPA II kit (AMS Biotechnology Europe, Oxfordshire, UK) as previously reported (Jabbour et al, 1998). Briefly, radiolabelled cRNA was generated using the linearised plasmid, T7 RNA polymerase and α^{32}P-UTP (800 Ci $mmol^{-1}$: Amersham, Buckinghamshire, UK). Total RNA (10 µg) from adenocarcinoma tissue and yeast ($n = 2$: used as reaction controls in the presence or absence of RNase digestion to establish the specificity of the hybridisation reaction and the size of the unprotected RNA fragment) was mixed with the radiolabelled probe (2×10^5 cpm) and hybridisation buffer, heated to 90˚C for 4 min and incubated overnight at 45˚C. Integrity of RNA and the relative amount of total RNA in each reaction was determined by including 18S radiolabelled cRNA in each reaction. Single-stranded RNA were digested using 250 U ml^{-1} RNase A and 10 000 U ml^{-1} RNase T1 for 30 min at 37˚C. The protected RNA was precipitated and separated on a 5% denaturing acrylamide gel. The gel was dried under vacuum and exposed to an autoradiographic film (XAR-5; Kodak).

Immunohistochemistry

Immunohistochemistry was performed on adenocarcinoma tissue ($n = 4$ of each of well, moderately and poorly differentiated), normal secretory phase endometrium ($n = 4$) and healthy post-menopausal uterus ($n = 4$). 5-µm paraffin way-embedded tissue sections were dewaxed in xylene, rehydrated in graded ethanol and washed in water followed by TBS (50 mM Tris-HCl, 150 mM NaCl pH 7.4) and blocked for endogenous peroxidase (3% H_2O_2 in methanol). Sections were blocked using either 20% normal rabbit serum (for COX-2), 20% swine serum (for mPGES, PGE_2 and EP4) or 20% normal goat serum (for CD34) diluted in TBS. Subsequently the tissue sections were incubated with polyclonal goat anti-COX-2 antibody (sc-1745; Autogenbioclear, Wilts, UK) at a dilution of 1:400, polyclonal rabbit anti-mPGES antibody (catalogue number 160140; Cayman Chemical, Alexis Corporation-Europe, Nottingham, UK) at a dilution of 1:50, polyclonal rabbit anti-EP4 receptor (catalogue number 101770; Cayman Chemical) at a dilution of 1:500, polyclonal rabbit anti-PGE_2 antibody (kindly supplied by Professor RW Kelly, MRC Human Reproductive Sciences Unit, Edinburgh, UK) at a dilution of 1:100 or monoclonal mouse anti-human CD34 primary antibody (mca-547; Serotec, Oxford, UK) at a dilution of 1:25 at 4˚C for 18 h. Control tissue was incubated with either 5% non-immune antisera (CD34), goat anti-COX-2 antibody pre-adsorbed to blocking peptide (sc-1745p; Autogenbioclear), rabbit anti-mPGES antibody pre-adsorbed to blocking peptide (catalogue number 360140: Cayman Chemical), rabbit anti-EP4 pre-adsorbed to blocking peptide (catalogue number 101780; Caman Chemical) or

rabbit anti-PGE_2 antibody pre-adsorbed to 10-fold excess PGE_2 (Sigma). After thorough washing with TBS, the tissue sections probed with the goat anti-human COX-2, rabbit anti-mPGES, rabbit anti-EP4 and rabbit anti-PGE_2 primary antibodies were incubated with biotinylated rabbit anti-goat secondary IgG antibody (for COX-2; Dako, Bucks, UK) or swine anti-rabbit secondary IgG antibody (for mPGES, EP4 and PGE_2; Dako) at a dilution of 1:500 for 40 min at 25˚C. Thereafter the tissue sections were incubated with streptavidin–biotin peroxidase complex (Dako) for 20 min at 25˚C. Tissue sections probed with the mouse anti-human CD34 antibody were developed using a Mouse En Vision Kit (Dako) as instructed by the manufacturer. Colour reaction was developed by incubation with 3.3′-diaminobenzidine (Dako).

Real time quantitative PCR

Endometrial RNA samples were extracted from adenocarcinoma tissue (n = 4 well differentiated, n = 6 moderately differentiated, n = 4 poorly differentiated) and normal secretory phase endometrium (n = 7) as described above. RNA samples were reverse transcribed using $MgCl_2$ (5.5 mM), dNTPs (0.5 mM each), random hexamers (2.5 µM). RNAase inhibitor (0.4 U μl^{-1}) and multiscribe reverse transcriptase (1.25 U μl^{-1}; all from PE Biosystems, Warrington, UK). The mix was aliquoted into individual tubes (16 µl $tube^{-1}$) and template RNA was added (4 µl $tube^{-1}$ of 100 ng μl^{-1} RNA). Samples were incubated for 60 min at 25˚C, 45 min at 48˚C and then at 95˚C for 5 min.

A reaction mix was made containing Taqman buffer (5.5 mM $MgCl_2$, 200 µM dATP. 200 µM dCTP, 200 µM dGTP, 400 µM dUTP), ribosomal 18S forward and reverse primers and probe (all at 50 nM), forward and reverse primers for EP receptor (300 nM), EP receptor probe (200 nM), AmpErase UNG (0.01 U μl^{-1}) and AmpliTaq Gold DNA Polymerase (0.025 U μl^{-1}; all from PE Biosystems). A volume of 48 µl of reaction mix was aliquoted into separate tubes for each cDNA sample and 2 µl $replicate^{-1}$ of cDNA was added. After mixing, 23 µl of sample were added to the wells on a PCR plate. Each sample was added in duplicate. A no template control (containing water) was included in triplicate. Wells were sealed with optical caps and the PCR reaction run on an ABI Prism 7700 using standard conditions. EP receptor primers and probe for quantitative PCR were designed using the PRIMER express program (PE Biosystems). The sequence of the EP2 receptor primers and probe were as follows; Forward: 5′-GAC CGC TTA CCT GCA GCT GTA C-3′; Reverse: 5′-TGA AGT TGC AGG CGA GCA-3′: Probe (FAM labelled): 5′-CCA CCC TGC TGC TGC TTC TCA TTG TCT-3′. The sequence of the EP4 receptor primers and probe were as follows; Forward: 5′-ACG CCG CCT ACT CCT ACA TG-3′; Reverse: 5′-AGA GGA CGG TGG CGA GAA T-3′; Probe (FAM labelled): 5′-ACG CGG GCT TCA GCT CCT TCC T-3′. The ribosomal 18S primers and probe sequences were as follows; Forward: 5′-CGG CTA CCA CAT CCA AGG AA-3′; Reverse: 5′-GCT GGA ATT ACC GCG GCT-3′; Probe (VIC labelled): 5′-TGC TGG CAC CAG ACT TGC CCT C-3′.

In vitro culture and cAMP measurement

Endometrial tumour tissue (n = 6; 2 well, 2 moderately and 2 poorly differentiated adenocarcinomas) and normal secretory phase endometrium (n = 6) were minced finely with scissors and incubated in 2 ml RPMI (Sigma) medium containing 10% fetal calf serum, 0.3 mg ml^{-1} L-glutamine, 100 IU penicillin, 100 µg streptomycin and 3 µg ml^{-1} indomethacin, for 1.5 h at 37 ˚C in humidified 5% CO_2. Thereafter samples were incubated in the same medium containing IBMX (Sigma) to a final concentration of 1 mM for 30 min at 37˚C and then stimulated for 5 min with 300 nM PGE_2. Control treatments received no PGE_2. Tissue was harvested by centrifugation at 2000 ***g***. The supernatant was discarded and the tissue homogenised in 0.1 M HCl. cAMP concentration was quantified by ELISA using a cAMP kit (Biomol: Affiniti, Exeter, UK) and normalised to protein concentration of the homogenate. Protein concentrations were determined using protein assay kits (Bio-Rad, Hemel Hempstead, UK). The data are presented as fold induction of cAMP following treatment with PGE_2. Fold induction was calculated by dividing cAMP values for the PGE_2-treated samples by the value for the untreated sample.

RESULTS

COX-2 expression in endometrial adenocarcinoma was assessed by ribonuclease protection assay. COX-2 expression was detected in well, moderately and poorly differentiated (n = 3 each grade) adenocarcinomas (Figure 1). No COX-2 expression was detected in the secretory phase endometrium collected from women with normal menstrual cycles (Figure 1). It was not possible to obtain fresh healthy post-menopausal endometrium to assess COX-2 RNA expression and hence secretory phase endometrium was chosen as a comparative tissue for the adenocarcinoma. This phase of the menstrual cycle was chosen as minimal proliferative activity is detected in the endometrium during the secretory phase (Ferenczy et al, 1979). This would be comparable to the absence of proliferative activity predicted in healthy post-menopausal endometrium.

Immunohistochemistry was employed in order to detect the site of expression of COX-2/mPGES and synthesis of PGE_2 in the endometrial adenocarcinomas. COX-2 and mPGES expression was detected in neoplastic epithelial cells in poorly, moderately and well differentiated adenocarcinoma (Figure 2A, 2C and 2E for COX-2 and Figure 3A, 3B and 3C for mPGES respectively). Minimal signal was detected in archival post-menopausal uterine specimens or secretory phase endometrium (Figures 2G, 2I, 3D and 3E) and no signal was detected when the antibody was pre-adsorbed with blocking peptide (Figures 2E and 3C insets). Similar to COX-2/mPGES, PGE_2 synthesis was detected in the

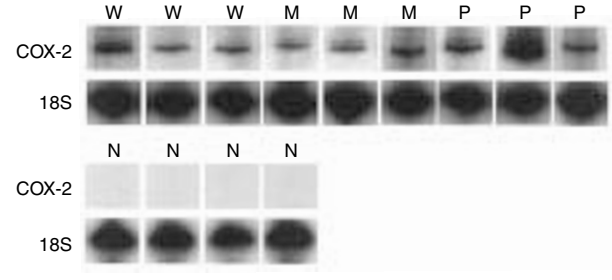

Figure 1 Ribonuclease protection assay conducted using 10 µg of total RNA extracted from normal secretory phase endometrium (N) and well (W), moderately (M) and poorly (P) differentiated endometrial adenocarcinoma tissue. COX-2 expression was detected using a 381 homologous cRNA probe. The integrity of the RNA and the relative amount of total RNA in each reaction were determined using a ribosomal 18S cDNA probe

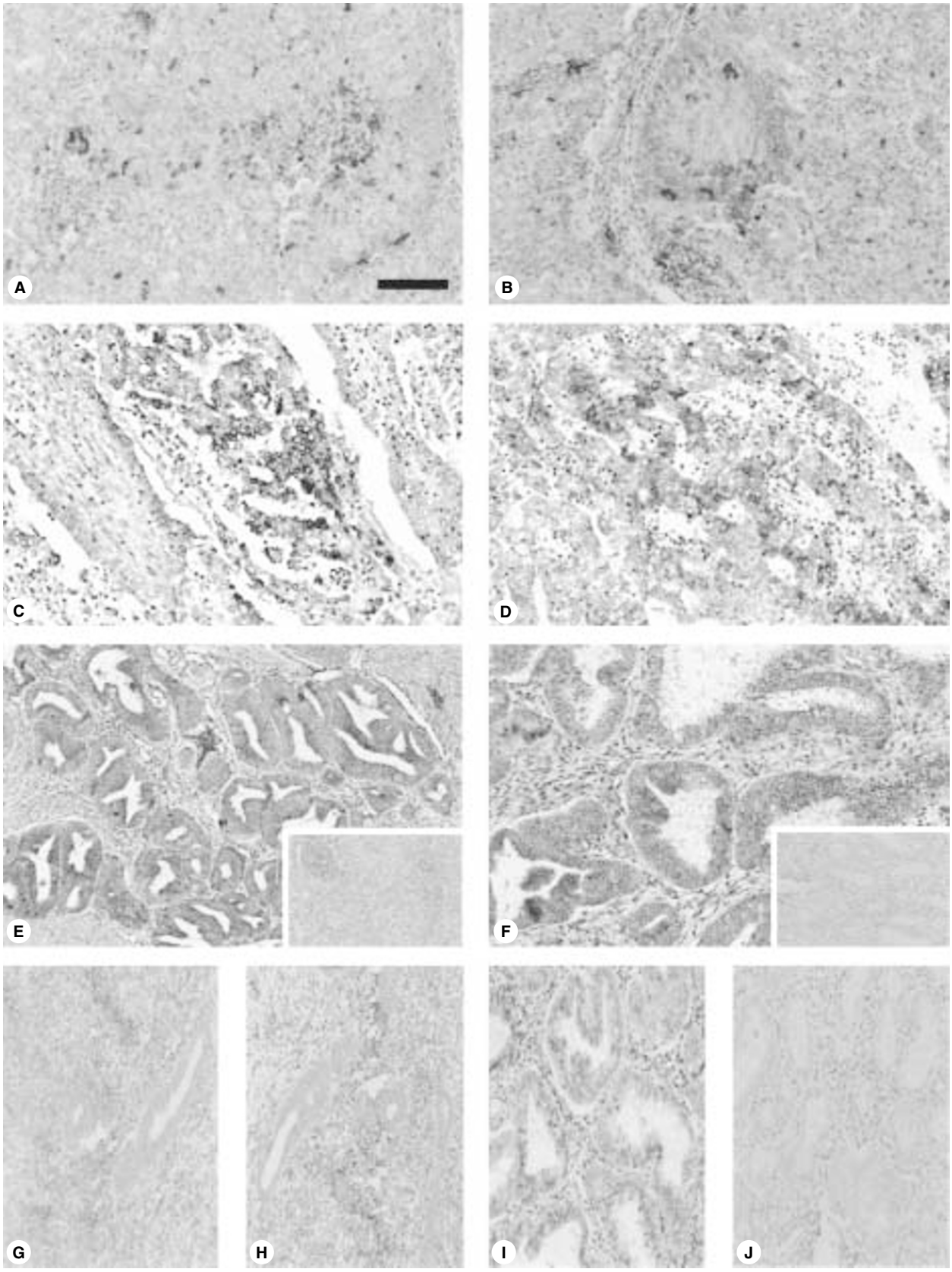

Figure 2 COX-2 expression and PGE_2 synthesis are detected in epithelial cells of poorly (**A** and **B** respectively), moderately (**C** and **D** respectively) and well (**E** and **F** respectively) differentiated endometrial adenocarcinoma. Minimal immunostaining for COX2 or PGE_2 were detected in post menopausal (**G** and **H**) or secretory phase (**I** and **J**) endometrium. Insets in (**E**) and (**F**) are sections that were stained with pre-adsorbed COX-2 and PGE_2 sera respectively (negative controls). Scale bar is 100 μm

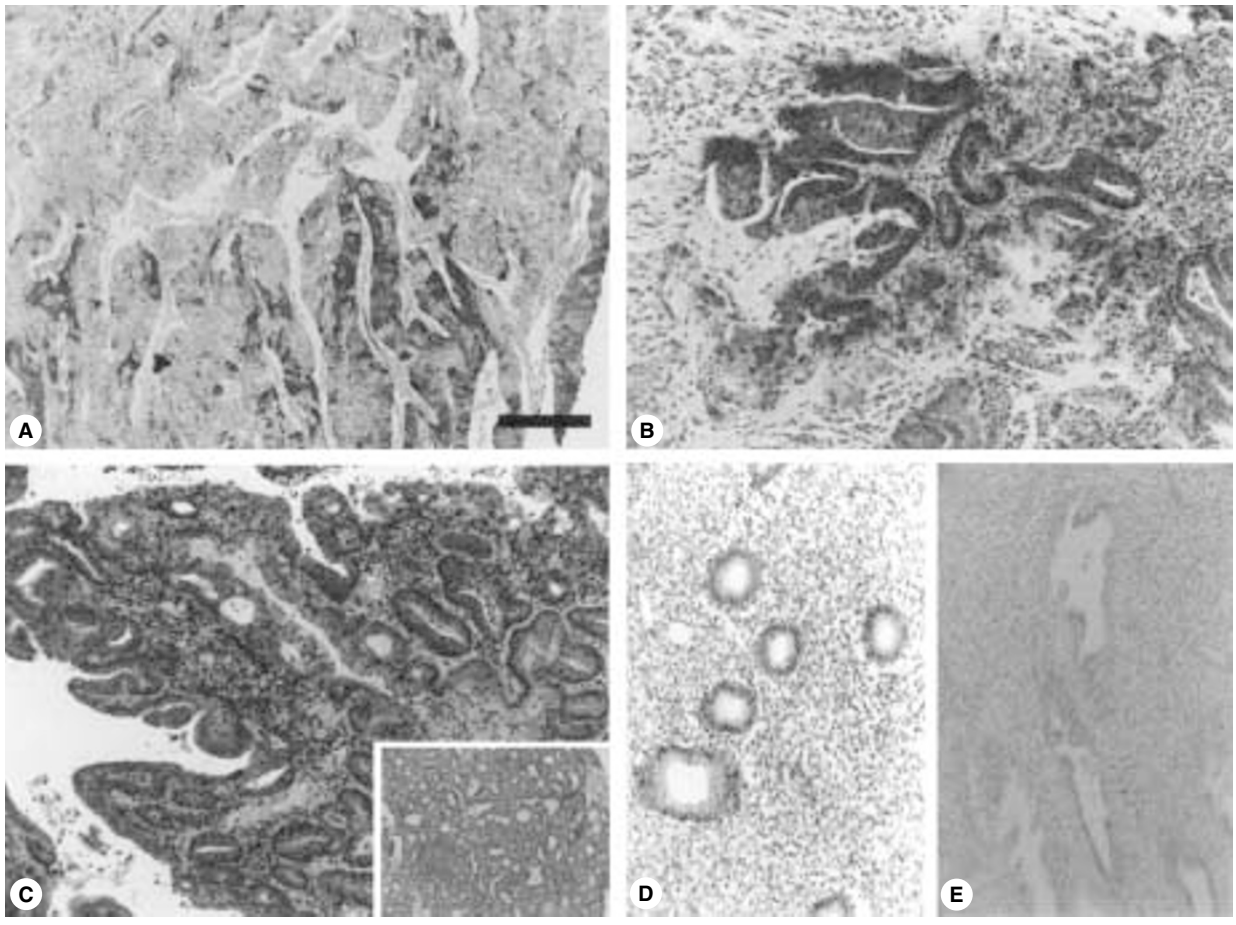

Figure 3 mPGES expression is detected in epithelial cells of poorly (**A**), moderately (**B**) and well (**C**) differentiated endometrial adenocarcinoma. Minimal immunostaining for mPGES was detected in post-menopausal uterus and secretory phase endometrium (**D** and **E** respectively). Inset in (**C**) is a section that was stained with mPGES pre-adsorbed serum (negative control). Scale bar is 100 μm

neoplastic epithelial cells of carcinomas of different grades of differentiation (Figures 2B, 2D and 2F respectively). Minimal immunostaining for PGE_2 was observed in post-menopausal uterus or secretory phase endometrium (Figures 2H and 2J respectively) and no staining was detected in tissue treated with pre-adsorbed sera (Figure 2F inset). In addition, COX-2, mPGES and PGE_2 immunostaining was observed in endothelial cells lining the vasculature in all adenocarcinoma sections investigated (Figures 4A, 4B and 4C respectively). To confirm that COX-2/mPGES expression and PGE_2 synthesis were localised to the endothelial cells of blood vessels, immunohistochemistry was performed on tissue sections using antibodies raised against the CD34 endothelial cell marker. The pattern of expression with CD34 (Figure 4e) was identical to that observed with COX-2, mPGES and PGE_2 thus confirming that COX-2/mPGES expression and PGE_2 synthesis are localised to the endothelial cell layer of blood vessels in human adenocarcinomas. Negligible staining was observed in the stromal compartment of all carcinoma tissue investigated.

The expression of 2 subtypes of PGE_2 receptors, namely EP2 and EP4, was investigated by real-time quantitative PCR in carcinoma tissue and normal secretory phase endometrium. Expression of both receptors was significantly up-regulated in adenocarcinoma tissues compared with normal secretory endometrium ($P < 0.01$). No differences in the level of expression of EP2 or EP4 receptors were detected between poorly, moderately or well differentiated adenocarcinomas (Figure 5A). Overall, the fold induction of EP2 and EP4 receptor expression in adenocarcinoma tissue (mean fold induction of all carcinoma samples) compared with normal secretory endometrium was 28.0 ± 7.4 and 52.5 ± 10.1 for EP2 and EP4 receptors respectively. Using immunohistochemistry, EP4 receptor expression was localised to neoplastic epithelial cells of carcinoma tissues of all grades of differentiation (Figure 5B) and also in endothelial cells of the microvasculature (Figure 4D).

In order to assess the activity of the EP2/EP4 receptors in the carcinoma tissue and normal secretory phase endometrium, cAMP generation was measured following short-term in vitro culture with or without PGE_2 (Figure 6). Comparable cAMP turnover in response to PGE_2 was observed in all carcinoma tissue. The fold induction of cAMP generation in response to PGE_2 was significantly higher in carcinoma tissue compared with secretory phase endometrium (3.42 ± 0.46 vs 1.15 ± 0.05 respectively; $P < 0.001$).

DISCUSSION

The data presented in this study demonstrate the expression of COX-2 and mPGES enzymes in adenocarcinomas of the uterus at different grades of differentiation as demonstrated by ribonuclease protection assays and immunohistochemistry. This is similar to a recent report by (Tong et al, 2000) in which heightened expression of COX-2, but not COX-1, was observed in endometrial adenocar-

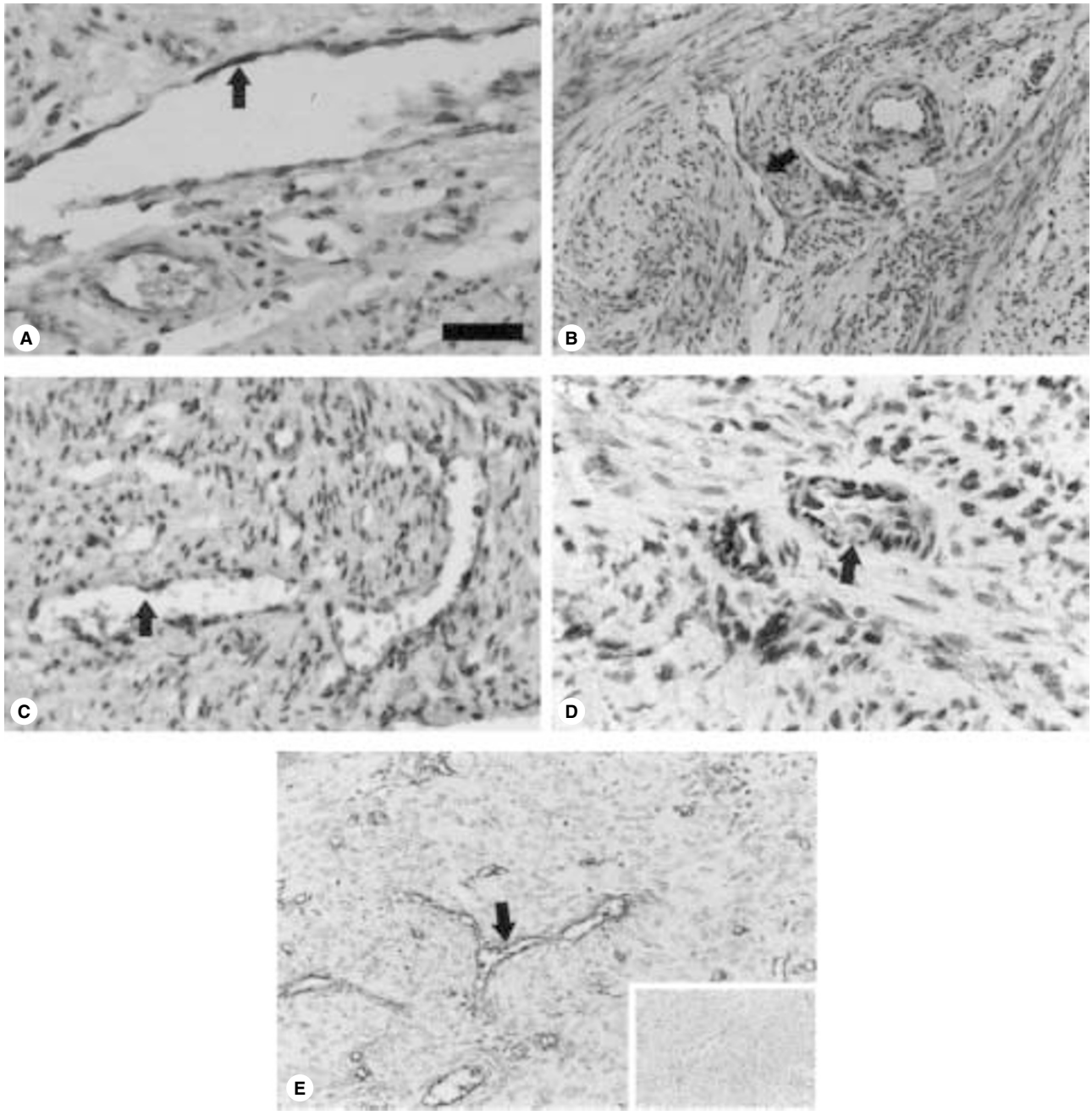

Figure 4 COX-2 (**A**), mPGES (**B**), PGE_2 (**C**) and EP4 (**D**) are detected in endothelial cells of all carcinoma tissues. Vascular endothelial cells in endometrial adenocarcinoma were localised using antibodies raised against the human CD34 endothelial cell marker (**E**). The inset in (**E**) is a section that was stained with non-immune goal serum (CD34 negative control). Negative controls for the other antibodies are presented in Figures 2, 3 and 5B. Scale bar is 50 μm

cinomas. COX-2 and mPGES expression were co-localised to neoplastic epithelial cells and endothelial cells of the microvasculature suggesting a co-regulated pattern of expression for the 2 genes. This is supported by recent evidence suggesting co-ordinate up- and down-regulation of mPGES and COX-2 (Thoren and Jakobsson, 2000). Previous studies have detected expression of mPGES in human smooth muscle vascular cells but not in umbilical vein endothelial cells although these cells retained synthetic capacity for PGE_2 (Soler et al, 2000). This apparent discrepancy may reflect tissue variation in regulation of expression of mPGES in endothelial cells. The over-expression of COX-2 enzyme observed in endometrial adenocarcinomas resembles that reported for a number of other carcinomas including colon, lung, bladder, stomach, pancreas, prostate and cervix (Tsujii et al, 1997; Wolii et al, 1998 Mohammed et al, 1999; Ratnasinghe et al, 1999; Tucker et al, 1999; Gupta et al, 2000; Sales et al, 2001). The exact intracel-

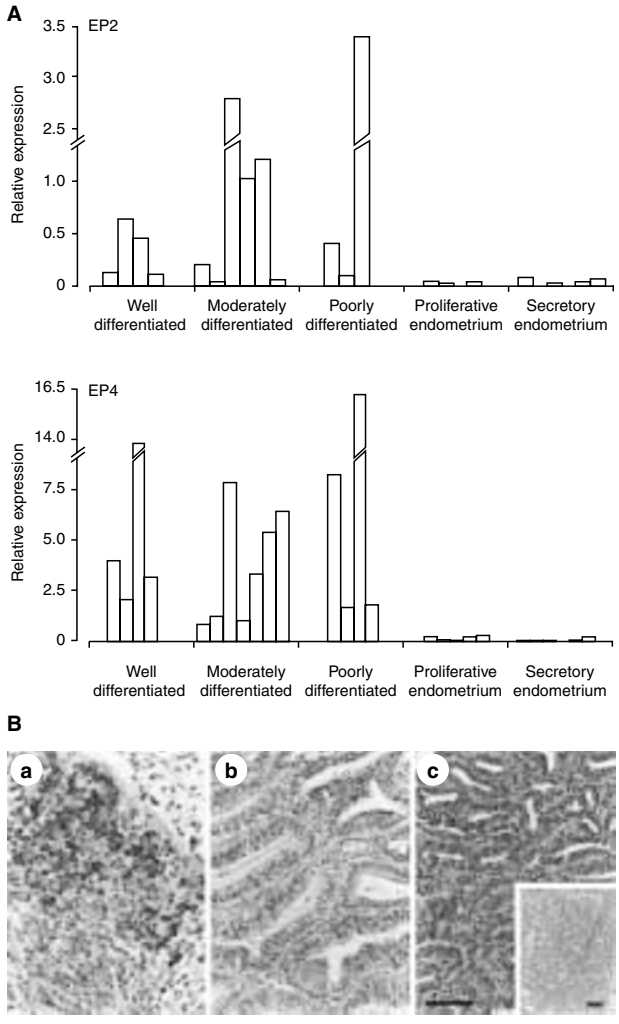

Figure 5 Relative expression of EP2 and EP4 receptors in endometrial adenocarcinoma of different grades of differentiation and in healthy secretory phase endometrium collected from fertile women with normal menstrual cycles. (**B**) EP4 receptor expression was detected in neoplastic epithelial cells of poorly (**a**), moderately (**b**) and well (**c**) differentiated uterine adenocarcinomas. The inset in (**c**) is a section that was stained with immune serum that had been pre-adsorbed with the blocking peptide. Scale bar is 50 μm

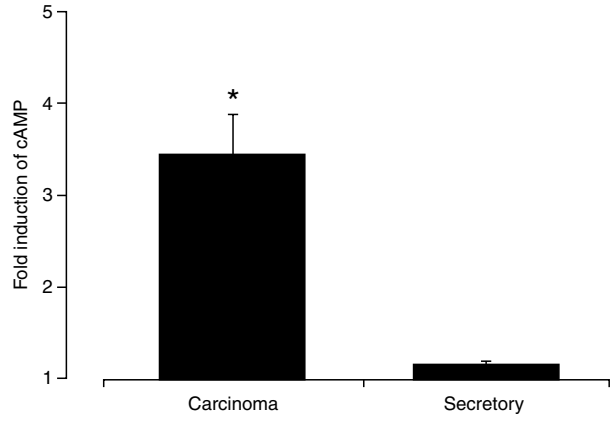

Figure 6 Fold induction of cAMP response in endometrial adenocarcinoma ($n = 6$) and healthy secretory phase endometrium ($n = 6$) following stimulation with 300 nM PGE_2. Fold induction was calculated by dividing cAMP values for the PGE_2-treated samples by the values for the untreated sample

lular signalling pathways that lead to up-regulation in COX-2 expression in carcinomas remain to be elucidated. However, recent data suggest regulatory roles for ERK2 MAP kinase (Jones et al, 1999a), p38 MAP kinase (Dean et al, 1999) and phosphatidylinositol 3-kinase (Weaver et al, 2001) in a number of model systems including endometrial adenocarcinoma epithelial cells (Munir et al, 2000).

The immunohistochemistry studies suggest that COX-2 and mPGES expression is associated with enhanced production of PGE_2 in neoplastic cells and endothelial cells of the microvasculature. Previous studies have shown that PGE_2 synthesis/secretion is significantly elevated in uterine carcinomas compared with normal uterus (Willman et al, 1976). Moreover, in a number of model systems PGE_2 synthesis and secretion are elevated in response to COX-2 up-regulation (Tsujii and DuBois, 1995). The biological role of COX-2 and PGE_2 in endometrial adenocarcinomas remains to be established. However, enhanced COX-2 expression and PGE_2 synthesis can induce neoplastic changes in epithelial cells through a number of biological pathways. These include promotion of cellular proliferation, inhibition of apoptosis, increasing metastatic potential of neoplastic cells and promoting angiogenesis (Rolland et al, 1980; Tsujii and DuBois, 1995; Tsuji et al, 1996). Over-expression of COX-2 enzyme in rat intestinal epithelial cells results in enhanced secretion of PGE_2 which is associated with increased cellular proliferation and resistance to apoptosis (Tsujii and DuBois, 1995). Similarly, over expression of COX-2 and increased production of prostaglandins have been linked to enhanced metastatic potential of neoplastic cells possibly through down-regulation of expression of cell adhesion molecules such as E-cadherin (Rolland et al, 1980). Expression of E-cadherin is down-regulated in a number of solid tumours and is closely and inversely related to enhanced invasion of neoplastically transformed cells (Schipper et al, 1991; Mayer et al, 1993).

Successful tumour establishment and metastasis is also dependent on initiation of angiogenesis at the site of growth of the tumour cells. COX-2 and PGE_2 are strongly linked with regulation of the angiogenic process during tumour development (Masferrer et al, 2000). Over-expression of COX-2 and increased production of PGE_2 in epithelial cells enhances the expression of angiogenic factors which act in a paracrine manner to induce endothelial cell migration and microvascular tube formation (Tsujii et al, 1998). Similarly, COX-2 and PGE_2 may influence angiogenesis directly by acting on endothelial cells. Treatment of endothelial cells with selective COX-2 inhibitors has been shown to reduce microvascular tube formation and this effect is partially reversed by co-treatment with PGE_2 (Jones et al, 1999b). Hence, it is feasible that in vivo angiogenesis in endometrial adenocarcinomas may be regulated by COX-2 and PGE_2 via an epithelial–endothelial and an endothelial–endothelial cell interaction. This is supported by the data presented in this study which localised the site of expression of COX-2, mPGES and PGE_2 to neoplastic epithelial cells and endothelial cells.

PGE_2 acts on target cells through interaction with 7 transmembrane G-protein coupled receptors. Different forms of the membrane-bound receptors have been cloned which utilise alternate intracellular signalling pathways. In this study we investigated the expression of 2 of the membrane-bound PGE_2 receptors, namely EP2 and EP4, which mediate their effect on target cells via the PKA pathway by activating adenylate cyclase and increasing

intracellular cAMP (Coleman et al, 1994). In endometrial adenocarcinoma, expression of EP2 and EP4 receptors is up-regulated in comparison with normal secretory phase endometrium and expression of at least the EP4 receptor is localised to neoplastic epithelial cells and the endothelium of the microvasculature. It was not possible to conduct parallel studies to localise EP2 receptors in the carcinoma tissues as no commercial antibodies are available for this receptor. Hence it remains to be established whether these receptors are co-expressed in the same cell type. However, using in situ hybridisation techniques, EP2 and EP4 receptor expression have been recently co-localised to epithelial and endothelial cells of the normal human endometrium (Milne et al, 2001). Functionality of the EP2/EP4 receptors in the carcinoma tissue was assessed by measuring cAMP generation following treatment with exogenous PGE_2. Treatment with PGE_2 resulted in a rapid cAMP generation thus demonstrating functional activation of the EP2 and/or EP4 receptors in this tissue. Up-regulation in expression and signalling of EP2/EP4 receptors has also been reported in cervical carcinoma and this suggests a common signalling pathway for PGE_2 in reproductive tract neoplasia (Sales et al, 2001). The exact role of COX-2/mPGES enzymes and PGE_2 and the associated EP2/EP4 receptors in endometrial adenocarcinoma remains to be established. However, it is reasonable to suggest that COX-2/mPGES and PGE_2 may mediate proliferation of epithelial and/or endothelial cells. The proliferating cells within endometrial adenocarcinomas are detected predominantly in post-menopausal women at a time when the healthy endometrium is expected to atrophy and display minimal cellular proliferation or angiogenesis. Moreover, healthy post-menopausal uterus and normal secretory endometrium, both of which have minimal proliferative or angiogenic activity, display negligible COX-2/mPGES/EP2/EP4 receptor expression and minimal cAMP generation in response to treatment with PGE_2. A possible role for PGE_2 in proliferation has already been established in a number of cell types including endothelial cells and it has been suggested that this effect is mediated via cAMP and induction of expression of mitogenic growth factors such as vascular endothelial growth factor and basic fibroblast growth factor (Hoper et al, 1997; Cheng et al, 1998). Future studies will elucidate the exact role of PGE_2 and its associated receptors on proliferation and neoplastic differentiation of epithelial/endothelial cells in endometrial adenocarcinomas.

In conclusion, these data confirm the expression of COX-2 and mPGES enzymes and synthesis of PGE_2 in endometrial adenocarcinoma of various grades of differentiation. Both COX-2/mPGES and PGE_2 are localised to the neoplastic epithelial cells and endothelial cells of the microvasculature. PGE_2 may exert an autocrine/paracrine effect in endometrial adenocarcinoma through interaction with EP2/EP4 receptors and activation of the PKA signalling pathway.

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