Adenoviral vectors. Replication-deficient *E1*- and *E3*-deleted Ad vectors were constructed using standard molecular cloning methods. Briefly, PCR was employed to generate a PCR product encoding the *15-PGDH* gene using human adult normal colon tissue cDNA (BioChain Institute, Hayward, CA) as template. To generate the pDrive-cox-PGDH plasmid the *15-PGDH* gene was cloned using *BspH* I and *Nhe* I sites into a plasmid pDrive-hCOX-2 (InvivoGen, San Diego, CA), which was digested and the *LacZ* gene was removed prior to cloning in the *15-PGDH* gene. To develop the pShuttle-cox-PGDH plasmid, a fragment including the *15-PGDH* open reading frame and SV40 late polyadenylation signal under control of the Cox-2 promoter element was replaced from pDrive-cox-PGDH using *Pst* I and *Swa* I, blunted using DNA Polymerase I Klenow and cloned into *EcoR* V digested pShuttle plasmid (Quantum Biotechnologies, Montreal, Canada). To generate the pGL3-PGDH plasmid a PCR product encoding the *15-PGDH* gene was cloned using *Hind* III and *Xba* I sites into the pGL3-Basic vector (Promega, Madison, WI), which was digested and the firefly luciferase (*Luc*) gene was removed prior to cloning in the *15-PGDH*. To generate the pShuttle-flt-PGDH plasmid, a fragment including the *15-PGDH* and SV40 late polyadenylation signal was replaced from the pGL3-PGDH using *Hind* III and *Sal* I and was incorporated into pShuttle-flt plasmid downstream of the flt-1 promoter element. To generate the pDrive-cox-Luc plasmid the firefly luciferase (*Luc*) gene was replaced from the pGL3-Basic using *Nco* I and *Xba* I, blunted using DNA Polymerase I Klenow and cloned into *BspH* I and *EcoR* I digested and blunted pDrive-hCOX-2 plasmid. The pDrive-cox-Luc plasmid was digested using *Bsp 120*I and *Swa* I restriction endonucleases, blunted and an assembled expression cassette including Cox-2 promoter, *Luc* open reading frame and the SV40 late polyadenylation signal was ligated to *EcoR* V digested pShuttle plasmid to generate pShuttle-cox-Luc vector. Recombinant Ad genomes were generated by homologous DNA

recombination in *E. coli* BJ5183 between pShuttle-flt-PGDH, pShuttle-cox-PGDH or pShuttle-cox-Luc and pAdEasy-1 plasmid (Quantum Biotechnologies). The newly generated genomes were confirmed by partial sequencing analysis, linearized with *Pac* I and transected into HEK293 cells using the SuperFect Transfection Reagent (QIAGEN, Chatsworth, CA) to generate Adflt-PGDH, Adcox-PGDH and Adcox-Luc recombinant Ads. Adflt-Luc (encoding *Luc* under control of the flt-1 promoter) and Adcmv-Luc (encoding *Luc* under control of the human cytomegalovirus (cmv) promoter element) were kindly provided by Dr. P. Reynolds (University of Adelaide, Adelaide, Australia). All viruses were propagated in HEK293 cells, purified by cesium chloride gradient ultracentrifugation, and subjected to dialysis. The quantity of Ad particles was monitored by absorbance of the dissociated virus at A_{260} nm. Viral titer was measured by a 50% tissue culture infectious dose ($TCID_{50}$) assay. Briefly, HEK293 cells were plated into 96-well plates at 5 x 10^3 cells/well, and allowed to adhere overnight. Next day, serial dilutions of the viral stock were added directly to cells. Cells were incubated for 10 days, and cytopathic effect was determined using a crystal violet staining assay. Cell culture medium was removed and surviving cells were then fixed and stained with 2% (w/v) crystal violet (Sigma-Aldrich) in 70% ethanol for 3 h at room temperature. The plates were washed extensively, air-dried and the ratio of positive wells with observable cytopathic effect for each viral preparation was determined. The viral titer was calculated by the Karber equation: $T = 10^{1+D(S-0.5)}/V$, where T is infectious titer in $TCID_{50}$/ml, D is the $\log_{10}$ of the dilution, S is the $\log_{10}$ for the initial dilution plus the sum of ratios, and V is the volume in ml of the diluted virus used for infection. Multiplicity of infection (MOI) for subsequent experiments was expressed as $TCID_{50}$ per cell.

Real-time quantitative RT-PCR. Quantitative analysis of EP receptors, Cox-2 and VEGFR1/flt-1 mRNA expression was performed using real-time RT-PCR. Cells were lysed in TRIzol

reagent (Invitrogen), total RNA was extracted using RNeasy Mini Kit (Qiagen), following standard protocol, and quantified spectrophotometrically using a MBA 2000 spectrophotometer (Perkin Elmer). cDNA was synthesized using random hexamer primers and an Omniscript RT kit (Qiagen). Primers and TaqMan probes (labeled at the 5' and 3' ends with 6-carboxyfluorescein (FAM) and 6-carboxy-tetramthyl-rhodamine (TAMRA) reporter dye, respectively) were designed using Primer Express Software (PE Applied Biosystems, Foster City, CA). For detection of the gene expression, the following forward (f), reverse (r) primers and TaqMan probe (pr) were used: EP1 f: AGT GCC AAG GGT GGT CCA A; EP1 r: CCG GGA ACT ACG CAG TGA CA; EP1 pr: TGG GCC TAA CCA AGA GTG CCT G; EP2 f: GAC CGC TTA CCT GCA GCT GTA C; EP2 r: TGA AGT TGC AGG CGA GCA; EP2 pr: CCA CCC TGC TGC TGC TTC TCA TTG TCT; EP3 f: AAG GCC ACG GCA TCT CAG T; EP3 r: TGA TCC CCA TAA GCT GAA TGG; EP3 pr: TCA ATC AGA TGT CGG TTG AGC AAT GCA A; EP4 f: ACG CCG CCT ACT CCT ACA TG; EP4 r: AGA GGA CGG TGG CGA GAA T; EP4 pr: ACG CGG GCT TCA GCT CCT TCC T; COX-2 f: GCT CAA ACA TGA TGT TTG ATT C; COX-2 r: GCT GGC CCT CGC TTA TGA; COX-2 pr: TGC CCA GCA CTT CAC GCA TCA GTT; 15-PGDH f: AAG CAA AAT GGA GGT GGA GGC; 15-PGDH r: TGG CAT TCA GTC TCA CAC CAC; 15-PGDH pr: CAT CTT TAG CAG GAC TCA TGC CCG TTG; flt-1 f: CAA TCA TCA TGG ACC CAG A; flt-1 r: CAC TTG CTG GCA TCA TAG G; flt-1 pr: CCT GGA TGA GCA GTG TGA ACG G. In each reaction, 20 ng of total cDNA was used as template and PCR was performed in 25 µl of reaction mixture containing 12.5 µl of 2x Taq Man Universal PCR Master Mix (PE Applied Biosystems), 300 nM each primer, and 100 nM fluorogenic probe. Amplifications were carried out in a 96-well reaction plate (PE Applied Biosystems) in a spectrofluorimetric thermal cycler (ABI PRISM 7000 Sequence Detector; PE

Applied Biosystems). After the initial denaturation (2 min at 95°C), amplification was performed with 45 cycles of 15 s at 95°C and 60 s at 60°C. Each sample was run in triplicate. A threshold cycle (C_t) for each triplicate was estimated by determining the point at which the fluorescence exceeded a threshold limit (10-fold the standard deviation of the baseline). Gene expression was normalized to RNA loading for each sample using GAPDH as an internal standard. The GAPDH primers and probe sequences were used as follows: GAPDH f: CCC ATG TTC GTC ATG GGT GT; GAPDH r: TGG TCA TGA GTC CTT CCA CGA TA; GAPDH pr: CTG CAC CAC CAA CTG CTT AGC ACC C. Results are expressed as relative expression to an internal standard RNA.

Table 1S. The levels of EP1-4 receptors, Cox-2, 15-PGDH and flt-1 mRNA expression

	MCF 10A	2LMP	MDA-MB-231	LCC6
EP1	2.6 ± 0.8	4.8 ± 1.1	6.0 ± 1.9	8.6 ± 2.5
EP2	0.6 ± 0.5	6.5 ± 2.8	8.3 ± 2.5	4.3 ± 2.1
EP3	1.1 ± 0.5	5.3 ± 1.7	4.5 ± 1.1	7.0 ± 1.9
EP4	2.8 ± 0.7	9.5 ± 3.0	3.9 ± 1.5	5.6 ± 1.7
Cox-2	2.3 ± 1.1	5.4 ± 1.2	7.2 ± 1.4	6.7 ± 1.7
15-PGDH	6.8 ± 2.5	2.3 ± 1.1	4.1 ± 1.3	4.3 ± 1.9
VEGFR1/flt-1	0.5 ± 0.4	14.4 ± 3.1	6.0 ± 2.9	11.2 ± 3.3

Relative levels of mRNA expression in comparison with GAPDH (internal standard).

FIGURE LEGENDS

Fig. 1S. Vascular staining in 2LMP xenograft tumors. Photomicrographs of immunocytochemistry staining of tumors from nude mice with 2LMP human breast cancer xenografts, using an anti-CD31 antibody (arrows). Representative areas of a 2LMP xenograft tumor from an animal treated with PBS (**A**), Adflt-Luc as viral control (**B**), Adcox-PGDH (**C**), Adflt-PGDH (**D**). Original magnification x200.

Fig. 2S. Microscopic examination of CD31-stained LS174T tumor sections. Microscopic examination of CD31-stained tumor sections from nude mice with LS174T human colon cancer xenografts (arrows). Representative areas of a LS174T xenograft tumor from an animal treated with PBS (**A**), Adflt-Luc alone (**B**), Adflt-PGDH alone (**C**), Adflt-Luc with Avastin (**D**), Adflt-PGDH in combination with Avastin (**E**). Original magnification x200.