ORIGINAL ARTICLE

COX-2 expression in ampullary carcinoma: correlation with angiogenesis process and clinicopathological variables

G Perrone, D Santini, A Verzi, B Vincenzi, D Borzomati, F Vecchio, R Coppola, A Antinori, P Magistrelli, G Tonini, C Rabitti

J Clin Pathol 2006;**59**:492–496. doi: 10.1136/jcp.2005.030098

See end of article for authors' affiliations

Correspondence to:
Dr Giuseppe Perrone,
Surgical Pathology,
Campus Bio-Medico
University, Via Emilio
Longoni 83, 00155 Rome,
Italy; g.perrone@
unicampus.it

Accepted for publication
27 September 2005

Background: There is evidence that the anti-neoplastic effect of non-steroidal anti-inflammatory drugs is attributable to cyclooxygenase-2 (COX-2) inhibition, but the exact mechanisms whereby COX-2 can promote tumour cell growth remain unclear. One hypothesis is the stimulation of tumour angiogenesis by the products of COX-2 activity. To date, there have been few clinicopathological studies on COX-2 expression in human ampullary carcinoma and no data have been reported about its relation with tumour angiogenesis.

Objective: To investigate by immunohistochemistry the expression of COX-2 and the angiogenesis process in a series of primary untreated ampullary carcinomas.

Methods: Tissue samples from 40 archival ampullary carcinomas were analysed for COX-2, vascular endothelial growth factor (VEGF), and an endothelial cell marker von Willebrand factor (vWF) by immunohistochemistry, using specific antibodies.

Results: COX-2 expression was detected in 39 tissue samples (97.5%), of which two (5%) were graded as weak, 26 (65%) as moderate, and 11 (27.5%) as strong. Only one lesion (2.5%) was negative for COX-2 expression. VEGF expression was detected in 36 tissue samples (90%). A significant positive correlation was found between COX-2 and VEGF expression. No statistical correlation was found between COX-2 expression and microvessel density.

Conclusions: COX-2 is highly expressed in ampullary carcinomas. This suggests an involvement of the COX-2 pathway in ampullary tumour associated angiogenesis, providing a rationale for targeting COX-2 in the treatment of ampullary cancer.

Carcinoma of the ampulla of Vater is a relatively uncommon neoplasm, accounting for approximately 6% of the tumours occurring in the region of head of pancreas and 0.2% of gastrointestinal tract malignancies.¹ Patients with cancer of the ampulla of Vater account for up to 36% of those undergoing surgery for pancreaticoduodenal malignancies and are the only patients among those affected by cancers of biliary-pancreatic origin who have up to a 50% chance of being cured by surgery alone.^{2–4} In addition, these tumours grow more slowly than cholangiocarcinomas and pancreatic adenocarcinomas.⁵

Recently, many studies have shown that ampullary and colon cancers are alike:

- ampullary carcinoma can arise from a pre-existing adenoma⁶;
- ampullary carcinomas often show the same histological appearances as colon cancer;
- patients with familial adenomatous polyposis (FAP) have a high prevalence of duodenal adenomas, and the region of the ampulla of Vater is the site of predilection for duodenal adenocarcinomas⁷;
- the molecular profile of ampullary carcinomas seems to be similar to colon cancer.⁸

The similar biological characteristics with colon carcinomas may partially explain their striking difference from pancreatic and biliary tract cancers in terms of growth behaviour and patient survival.

Epidemiological studies indicate that use of aspirin and other non-steroidal anti-inflammatory drugs—the major target of which is cyclooxygenase (COX), the rate limiting

enzyme converting arachidonic acid to prostaglandins—decreases the incidence and mortality from colorectal, gastric, and oesophageal cancers.^{9–11}

The exact mechanisms by which COX-2 overexpression can promote tumour cell growth are still unclear. Several molecular pathways have been hypothesised. One hypothesis is the stimulation of tumour angiogenesis by the products of COX-2 activity.^{12–13}

Angiogenesis plays a central role in the survival of cancer cells, in local tumour growth, and in the development of distant metastasis.¹⁴ Massive formation of blood vessels at the tumour site increases the opportunity for tumour cells to enter the circulation. Thus microvessel density (MVD) is considered to influence tumour metastasis and consequently prognosis in various human cancers.¹⁵ Vascular endothelial growth factor (VEGF) is a well characterised angiogenic factor and is known to play a crucial role in neovascularisation and in cancer progression.¹⁶ The best characterised factor involved in the regulation of VEGF is hypoxia, which has been shown to increase transcription of the gene and to stabilise its mRNA product.¹⁷ Furthermore, hypoxia upregulates COX-2, which increases the conversion of prostaglandin E₂ (PGE₂) from arachidonic acid. PGE₂ then induces entry of hypoxia inducible factor-1 α (HIF-1 α) from the cytosol into the nucleus, inducing transcription of VEGF.¹⁸ In this way, COX-2 is able to induce the transcription and the expression of VEGF protein.

Abbreviations: COX-2, cyclooxygenase-2; HIF, hypoxia inducible factor; HIS, immunohistochemical score; IQR, interquartile range; MVD, microvessel density; PGE₂, prostaglandin E₂; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor

Table 1 Clinicopathological indices of the study patients

Total number	40
Median age (range)	64.5 years (38 to 78)
Sex	
Male	22 (55%)
Female	18 (45%)
T factor	
1	7 (17.5%)
2	19 (47.5%)
3	13 (32.5%)
4	1 (2.5%)
N factor	
0	23 (57.5%)
1	17 (42.5%)

To gain further insight into the role of COX-2 and VEGF in ampullary carcinomas, we investigated their expression in a series of 40 ampullary cancers. Moreover, to the best of our knowledge for the first time, we examined the possible relations between COX-2, VEGF, and MVD in ampullary cancer.

METHODS

Clinical data and tumour specimen acquisition

The material included 40 surgical specimens of invasive ampullary carcinomas. All patients were staged by thoracic, abdominal, and pelvic computed tomography, and, when indicated, by intraoperative ultrasound of the liver to exclude the presence of distant metastases. Pathological findings were obtained from the pathologists' original reports. In

Figure 1 Vascular endothelial growth factor (VEGF) expression was detected in 90% of samples; 10% were assessed as negative (A), 30% as weak (B), 47.5% as moderate (C), and 12.5% as strong (D). Staining was cytoplasmatic, with expression localised to the tumour cells. Cyclooxygenase-2 (COX-2) expression was detected in 97.5% of samples, of which 5% were graded weak (F), 65% moderate (G), and 27.5% as strong (H). Only one lesion was negative for COX-2 expression (E). Staining was cytoplasmatic and granular, and localised to the tumour cells and not the surrounding stroma. (Original magnification $\times 400$.)

Table 2 Correlation between clinicopathological and immunohistochemical parameters

	No	COX-2 positive	p Value*	VEGF positive	p Value*	MVD (median (IQR))	p Value†
Age (years)							
≤ 50	5	5		5		72 (54.0 to 73.0)	
> 50	35	34	1.000	31	1.000	42 (32.5 to 65.5)	0.220
Sex							
Male	22	22		20		40 (31.5 to 59.2)	
Female	18	17	0.450	16	1.000	64 (36.7 to 73.0)	0.112
T factor							
1	7	7		6		42 (32.5 to 64.0)	
2	19	19	1.000	18	1.000	54 (38.5 to 66.5)	0.563
3	13	13	1.000	12	1.000	50 (31.0 to 68.0)	0.712
4	1	0	0.071	0	0.143	15	0.106
N factor							
0	23	23		21		43 (36.5 to 66.0)	
1	17	16	0.425	15	1.000	54 (30.0 to 68.0)	0.661

*Fisher's exact test.

†Mann-Whitney U test.

COX, cyclooxygenase; IQR, interquartile range; MVD, microvessel density; T, N, tumour, node classification; VEGF, vascular endothelial growth factor.

addition to the original pathology reports, microscopic findings (differentiation status and TNM classification) were reassessed. Tumours were categorised as per the International Union Against Cancer.¹⁹

Immunohistochemical staining and quantification

Immunohistochemical staining was carried out using the streptavidin-biotin method. Immunostaining involved the use of a rabbit antibody against VEGF protein (A-20: Santa Cruz Biotechnology, Santa Cruz, California, USA), a rabbit antibody against vWF protein (Dakocytomation A/S, Glostrup, Denmark), and a goat antibody against COX-2 protein (M-19: Santa Cruz Biotechnology). Slides were examined by two independent pathologists (GP and AV) blinded to each other's work and with no prior knowledge of clinical or pathological findings. Staining score was evaluated as the percentage ratio of stained cells to the total number of cell evaluated. On the basis of previous experience,²⁰ interpretation of staining score for VEGF was defined as positive when >10% of tumour cells stained, and negative when none or ≤10% of tumour cells stained. Furthermore, those tumours defined as VEGF positive on the base of the percentage ratio of stained cells, were regraded according to the intensity of staining using a scale of 1–3, where 1 represents mild, 2 moderate, and 3 strong intensity of staining.

Figure 2 Vascular endothelial growth factor (VEGF) grade 1 tumours were characterised by a lower MVD than VEGF grade 2 tumours ($p=0.001$), and VEGF grade 2 tumours by a lower MVD than VEGF grade 3 tumours ($p=0.012$).

For the evaluation of COX-2 expression we used an immunohistochemical score (IHS) calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score), as follows: no staining was scored as 0; 1–10% of cells stained scored as 1; 11–50% as 2; 51–80% as 3; and 81–100% as 4. Staining intensity was rated on a scale of 0 to 3, with 0 = negative; 1 = weak; 2 = moderate, and 3 = strong. The raw data were converted to the IHS score by multiplying the quantity and staining intensity scores. Theoretically, the scores could range from 0 to 12. An IHS score of 9–12 was considered strong immunoreactivity, 5–8 was considered moderate, 1–4 was considered weak, and 0 was scored as negative.²¹

Determination of MVD was carried out by hot spot analysis using Weidner's method. A detailed description of the immunohistochemical evaluation of MVD was given in our previous report.²⁰ Negative control slides processed without primary antibody were included for each staining run.

Statistical analysis

Spearman's rank correlation or Fischer's exact test were used to assess relations between ordinal data. MVD value between different groups were compared using the Mann-Whitney U test for non-parametric independent variables. Probability (p) values ≤0.05 were regarded as statistically significant in two tailed tests. SPSS software (version 10.00, SPSS Inc, Chicago, Illinois, USA) was used for the statistical analyses.

Table 3 Correlation matrix between immunohistochemical indices*

	COX-2	VEGF	MVD
COX-2		$R=0.496$ $p=0.001$	$R=0.308$ $p=0.053$
VEGF	$R=0.496$ $p=0.001$		$R=0.741$ $p<0.0001$
MVD	$R=0.308$ $p=0.053$	$R=0.741$ $p<0.0001$	

*Spearman test, R , correlation coefficient.

COX, cyclooxygenase; MVD, microvessel density; VEGF, vascular endothelial growth factor.

RESULTS

Patient characteristics and histological classification

The cohort consisted of 40 patients with a pathological diagnosis of cancer of the ampulla (22 men, 18 women), in all cases radically resected. The median age at diagnosis was 64.5 years (range 38 to 78). Seven patients (17.5%) were classified as T1, 19 (47.5%) as T2, 13 (32.5%) as T3, and only one (2.5%) as T4. Seventeen patients (42.5%) had lymph node metastases (table 1).

Immunohistochemical staining for VEGF and COX-2

VEGF expression (fig 1, panels A to D) was detected in 36 of the 40 samples (90%). Among 40 lesions, four (10%) were assessed as negative, 12 (30%) as weak, 19 (47.5%) as moderate, and five (12.5%) as strong. COX-2 expression (fig 1, panels E to H) was detected by semiquantitative scoring in 39 of the 40 samples (97.5%), of which, two (5%) were graded weak, 26 (65%) moderate, and 11 (27.5%) strong. Only one lesion (2.5%) was negative for COX-2 expression.

Correlations between immunohistochemical and pathological variables and patient characteristics

No significant correlations were identified between pathological (T and N) and patient characteristics (age and sex). No correlations were found between immunohistochemical indices (COX-2, MVD, and VEGF), patient characteristics, and T and N factors (table 2).

Relation between MVD and VEGF expression

A strong positive statistical correlation was found between VEGF expression score and MVD ($p < 0.0001$) (table 3). The median value of MVD of all cases studied was 46.5 (IQR, 32.75 to 67). MVD was lower in VEGF negative than in VEGF positive tumours (median 24.5 (IQR 21.75 to 29.25) ν 57.5 (37.25 to 67.25)) ($p = 0.02$). Moreover, VEGF grade 1 tumours were characterised by a lower MVD than VEGF grade 2 tumours (median 32.5 (IQR 25.75 to 39.25) ν 61 (42.5 to 67)) ($p = 0.001$), and VEGF grade 2 tumours by a lower MVD than VEGF grade 3 tumours (median 61 (IQR 42.5 to 67) ν 73 (72 to 83)) ($p = 0.012$) (fig 2; table 4).

Correlation between COX-2, VEGF expression, and MVD

A positive correlation was found between COX-2 and VEGF expression ($p = 0.001$) (table 5). No correlation was found between COX-2 expression score and MVD ($p = 0.053$) (table 3). Moreover, no difference was found in median value of MVD if tumours were distinguished on the basis of COX-2 expression score (table 4).

Table 4 Relation between microvessel density and vascular endothelial growth factor and COX-2 expression

	Grade	No	MVD (median (IQR))	p Value*
VEGF	0	4	24.5 (21.75 to 29.25)	
	1	12	32.5 (25.75 to 39.25)	0.332
	2	19	61 (42.5 to 67)	0.001
	3	5	73 (72 to 83)	0.012
COX-2	0	1	15	
	1	2	52.5 (46.25 to 58.75)	0.221
	2	26	41 (31.25 to 64.25)	0.623
	3	11	66 (48 to 72.5)	0.093

*Mann-Whitney U test.

COX, cyclooxygenase; IQR, interquartile range; MVD, microvessel density; VEGF, vascular endothelial growth factor.

Table 5 Relation between COX-2 and vascular endothelial growth factor expression

COX-2	VEGF				Total
	0	1	2	3	
0	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1
1	0 (0%)	1 (50%)	1 (50%)	0 (0%)	2
2	3 (11.5%)	10 (42.3%)	12 (46.1%)	1 (3.8%)	26
3	0 (0%)	1 (9%)	6 (54.5%)	4 (36.4%)	11
Total	4	12	19	5	40

Spearman test, $r = 0.496$; $p = 0.001$.

COX, cyclooxygenase; VEGF, vascular endothelial growth factor.

DISCUSSION

To date, there have been few studies on COX-2 expression in human ampullary cancer tissues. In our series the great majority of ampullary carcinomas (97.5%) showed expression of COX-2 protein, suggesting an important role in ampullary cancer.

Recent studies have shown that COX-2 is involved in several potential mechanisms of cancer development and progression. COX-2 inhibits apoptosis by activation of an apoptosis repressor gene, *bcl-2*^{22, 23}; confers invasive ability on tumour cells *in vitro* by activating metalloproteinase, which is the prerequisite for tumour invasion²⁴; and stimulates tumour angiogenesis through the modulation of production of variable angiogenic factors.^{16, 22}

Tumour angiogenesis is controlled by a balance of angiogenic and angiostatic regulators involved in multiple pathways that result in endothelial proliferation, differentiation, and organisation into a functional network of vascular channels.^{25, 26} Moreover, tumour angiogenesis, as quantitated by measurement of MVD, has been shown to be a significant negative prognostic factor in several cancers.^{27–29} Among the many reported angiogenic factors, VEGF—a key factor for induction of tumour angiogenesis—is increased in various human tumours.^{20, 30} In the present study, a positive statistical correlation was found between VEGF and MVD in ampullary carcinoma ($p < 0.0001$). The median value of MVD was lower in VEGF negative than in VEGF positive tumours ($p = 0.02$). These data are consistent with other reports that found a strong positive correlation between VEGF expression and MVD in various human tumours.^{31, 32} We believe that our study has shown for the first time that there is a relation between VEGF expression and MVD in human ampullary carcinoma. Furthermore, on the basis of the median MVD value, a significant statistical difference was found between VEGF grade 1 and grade 2 tumours ($p = 0.001$) and between VEGF grade 2 and grade 3 tumours ($p = 0.012$). These results show the direct relation between MVD and VEGF as any augmentation of VEGF expression is associated with a significant increase in MVD.

Tsujii *et al*¹⁶ used an endothelial cell/colon carcinoma co-culture model to explore the role of COX in tumour related angiogenesis. They showed that COX-2 overexpression stimulates endothelial motility and tube formation by the increased production of proangiogenic factors such as VEGF. These effects can be blocked by NS-398, a selective inhibitor of COX-2. One hypothesis is the stimulation of tumour angiogenesis by the products of COX-2 activity, especially PGE2. PGE2 has been reported to be capable of stimulating VEGF production in various cell types,^{33–35} and to enhance angiogenesis in pancreatic carcinoma.³⁶

To investigate the angiogenic pathway involved in COX-2 activity in ampullary cancer, we evaluated VEGF expression and found that it was correlated with COX-2 immunoreactivity ($p = 0.001$). To the best of our knowledge, this is the

Take home messages

- COX-2 is highly expressed in ampullary carcinomas, suggesting involvement of the COX-2 pathway in ampullary tumour associated angiogenesis.
- This provides a rationale for targeting COX-2 in the treatment of ampullary cancer.

first time a relation has been shown between COX-2 activity and VEGF expression in human ampullary carcinoma.

These data are in accordance with previous *in vitro* studies in which hypoxia—a common event in tumour biology resulting from exponential cellular proliferation³⁷—upregulates COX-2 expression, which increases PGE2 levels; PGE2 induces translocation of HIF-1 α into the nucleus, inducing VEGF transcription.¹⁸ COX-2-induced prostaglandins contribute to tumour growth by promoting the formation of new blood vessels that sustain tumour cell viability and growth.³⁸ Moreover, recent data indicate that COX-2 inhibitors are powerful anti-angiogenic agents *in vivo*.¹⁶

In our series, we failed to demonstrate a correlation between COX-2 expression and tumour MVD ($p = 0.053$). COX-2 expression and MVD were not directly correlated, so any augmentation of COX-2 expression associated with increase in MVD probably results from its strong relation to VEGF expression.

Our immunohistochemical findings cannot show a direct cause–effect link between COX-2 overexpression and tumour angiogenesis through VEGF activity. However, our results suggest an involvement of the COX-2 pathway in ampullary tumour associated angiogenesis and provide the rationale for clinical studies aimed at examining the efficacy of COX-2 inhibitors for the treatment or prevention of ampullary carcinoma.

ACKNOWLEDGEMENTS

We thank Alessandra Innocenzi and Giorgio Lescarini for technical support and Flavia Mancuso and Maria Crapulli for useful collaboration.

Authors' affiliations

G Perrone, A Verzi, C Rabitti, Surgical Pathology, Campus Bio-Medico University, Rome, Italy

D Santini, B Vincenzi, G Tonini, Oncology Unit, Campus Bio-Medico University, Rome, Italy

D Borzomati, R Coppola, General Surgery Department, Campus Bio-Medico University, Rome, Italy

F Vecchio, Anatomic Pathology And Histology, Catholic University of Sacred Heart, Rome, Italy

A Antinori, P Magistrelli, General Surgery Department, Catholic University of the Sacred Heart, Rome, Italy

REFERENCES

- Brennan MF. Surgical management of peripancratic cancer. In: Bland KL, Karakousis CP, Copeland EM, editors. *Atlas of surgical oncology*. Philadelphia: WB Saunders, 1995:473–85.
- Klempnauer J, Ridder GJ, Pichlmayr R. Prognostic factors after resection of ampullary carcinoma: multivariate survival analysis in comparison with ductal cancer of the pancreatic head. *Br J Surg* 1995;**82**:1686–91.
- Neoptolemos JP, Talbot IC, Shaw DC, et al. Long-term survival after resection of ampullary carcinoma is associated independently with tumor grade and a new staging classification that assesses local invasiveness. *Cancer* 1988;**61**:1403–7.
- Roder JD, Schneider PM, Stein HJ, et al. Number of lymph node metastases is significantly associated with survival in patients with radically resected carcinoma of the ampulla of Vater. *Br J Surg* 1995;**82**:1693–6.
- Kim HJ, Sohn TS, Lee KT, et al. Expression of cyclooxygenase-2 and its correlation with clinicopathologic factors of ampulla of Vater cancer. *J Korean Med Sci* 2003;**18**:218–24.
- Beger HG, Treitschke F, Gansauge F, et al. Tumor of the ampulla of Vater: experience with local or radical resection in 171 consecutively treated patients. *Arch Surg* 1999;**134**:526–32.
- Björk J, Akerbrant H, Iselius L, et al. Periapillary adenomas and adenocarcinomas in familial adenomatous polyposis: cumulative risks and APC gene mutations. *Gastroenterology* 2001;**121**:1127–35.
- Esposito I, Friess H, Buchler MW. Carcinogenesis of cancer of the papilla and ampulla: pathophysiological facts and molecular biological mechanisms. *Langenbecks Arch Surg* 2001;**386**:163–71.
- Thun MJ, Namboodiri MM, Heath CW. Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med* 1991;**325**:1593–6.
- Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* 1994;**5**:138–46.
- Thun MJ. NSAID use and decreased risk of gastrointestinal cancers. *Gastroenterol Clin North Am* 1996;**25**:333–48.
- Ziche M, Jones J, Gullino PM. Role of prostaglandin E1 and copper in angiogenesis. *J Natl Cancer Inst* 1982;**69**:475–82.
- Seed MP, Brown JR, Freemantle CN, et al. The inhibition of colon-26 adenocarcinoma development and angiogenesis by topical diclofenac in 2.5% hyaluronan. *Cancer Res* 1997;**57**:1625–9.
- Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990;**82**:4–6.
- Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991;**64**:327–36.
- Tsuji M, Kawano S, Tsuji S, et al. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 1998;**93**:705–16.
- Liu LX, Lu H, Luo Y, et al. Stabilization of vascular endothelial growth factor mRNA by hypoxia-inducible factor 1. *Biochem Biophys Res Commun* 2002;**291**:908–14.
- Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E2 induces hypoxia-inducible factor-1 α stabilization and nuclear localization in a human prostate cancer cell line. *J Biol Chem* 2002;**277**:50081–6.
- Sobin LH, Wittekind C. *TNM classification of malignant tumours*, 6th edition. UICC International Union Against Cancer. New York: Wiley-Liss, 2002.
- Perrone G, Vincenzi B, Santini D, et al. Correlation of p53 and bcl-2 expression with vascular endothelial growth factor (VEGF), microvessel density (MVD) and clinico-pathological features in colon cancer. *Cancer Lett* 2004;**208**:227–34.
- Soslow RA, Dannenberg AJ, Rush, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer* 2000;**89**:2637–45.
- Tsuji M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995;**83**:493–501.
- Sheng H, Shao J, Morrow JD, et al. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res* 1998;**58**:362–6.
- Tsuji M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci USA* 1997;**94**:3336–40.
- Folkman J. Clinical applications of research on angiogenesis. *N Engl J Med* 1995;**333**:1757–63.
- Risau W. Mechanisms of angiogenesis. *Nature* 1997;**386**:671–4.
- Weidner N, Semple JP, Welch WR, et al. Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* 1991;**324**:1–8.
- Macchiarini P, Fontanini G, Hardin MJ, et al. Relation of neovascularisation to metastasis of non-small-cell lung cancer. *Lancet* 1992;**340**:145–6.
- Weidner N, Carroll PR, Flax J, et al. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 1993;**143**:401–9.
- Kolch W, Martiny-Baron G, Kieser A, et al. Regulation of the expression of the VEGF/VPS and its receptors: role in tumor angiogenesis. *Breast Cancer Res Treat* 1995;**36**:139–55.
- Ferrara N, Heinsohn H, Walder CE, et al. The regulation of blood vessel growth by vascular endothelial growth factor. *Ann NY Acad Sci* 1995;**752**:246–56.
- Leung DW, Cachianes G, Kuang WJ, et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989;**246**:1306–9.
- Inoue H, Takamori M, Shimoyama Y, et al. Regulation by PGE2 of the production of interleukin-6, macrophage colony stimulating factor, and vascular endothelial growth factor in human synovial fibroblasts. *Br J Pharmacol* 2002;**136**:287–95.
- Mukutmoni M, Hubbard NE, Erickson KL. Prostaglandin E(2) modulation of vascular endothelial growth factor production in murine macrophages. *Prostaglandins Leukot Essent Fatty Acids* 2001;**65**:123–31.
- Pai R, Szabo IL, Soreghan BA, et al. PGE(2) stimulates VEGF expression in endothelial cells via ERK2/JNK1 signaling pathways. *Biochem Biophys Res Commun* 2001;**286**:923–8.
- Eibl G, Takata Y, Boros LG, et al. Growth stimulation of COX-2-negative pancreatic cancer by a selective COX-2 inhibitor. *Cancer Res* 2005;**65**:982–90.
- Paul SA, Simons JW, Mabeesh NJ. HIF at the crossroads between ischemia and carcinogenesis. *J Cell Physiol* 2004;**200**:20–30.
- Atiqur Rahman M, Toi M. Anti-angiogenic therapy in breast cancer. *Biomed Pharmacother* 2003;**57**:463–70.