Cellular Oncology 29 (2007) 477–482
IOS Press

EGF-R is expressed and AP-1 and NF-κB are activated in stromal myofibroblasts surrounding colon adenocarcinomas paralleling expression of COX-2 and VEGF

Panagiotis A. Konstantinopoulos [a,b,*], Gerasimos P. Vandoros [c,*], Michalis V. Karamouzis [a,d], Maria Gkermpesi [c], Georgia Sotiropoulou-Bonikou [e] and Athanasios G. Papavassiliou [a,**]

[a] *Department of Biological Chemistry, Medical School, University of Athens, Athens, Greece*
[b] *Division of Hematology–Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*
[c] *Department of Pathology, Aeghion General Hospital, Aeghion, Greece*
[d] *Division of Hematology–Oncology, University of Pittsburgh, Pittsburgh, PA, USA*
[e] *Department of Anatomy and Histology–Embryology, School of Medicine, University of Patras, Patras, Greece*

Abstract. *Background*: COX-2 and VEGF are important triggers of colon cancer growth, metastasis and angiogenesis. *Cox-2* promoter contains transcriptional regulatory elements for AP-1 and NF-κB transcription factors whilst *vegf* is a known AP-1 downstream target gene. We investigated whether stromal myofibroblasts surrounding colon adenocarcinomas express COX-2 and VEGF and whether activation of AP-1 and NF-κB, as well as expression of EGF-R parallel expression of COX-2 and VEGF in these cells. *Methods*: Immunohistochemical methodology was performed on archival sections from 40 patients with colon adenocarcinomas. We evaluated c-FOS, p-c-JUN (phosphorylated c-JUN), p-IκB-α (phosphorylated IκB-α), EGF-R, COX-2, NF-κB and VEGF expression in stromal myofibroblasts surrounding colon adenocarcinomas. Double immunostaining with a-smooth muscle actin and each antibody was done to verify the expression of these molecules in stromal myofibroblasts. *Results*: VEGF, p-IκB-α, NF-κB, c-FOS, p-c-JUN, EGF-R and COX-2 were expressed in stromal myofibroblasts surrounding colon adenocarcinomas in the majority of cases. EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN correlated positively with COX-2 and VEGF expression. *Conclusion*: Stromal myofibroblasts surrounding colon adenocarcinomas are an important source of VEGF and COX-2 production, while AP-1 and NF-κB transcription factors are activated and EGF-R is expressed in these cells and associated with COX-2 and VEGF production.

Keywords: Colon adenocarcinoma, stromal myofibroblasts, EGF-R, AP-1, NF-κB, COX-2, VEGF

1. Introduction

Several studies in breast, colon and prostate carcinomas indicate that carcinogenesis is associated with progressive alterations in the functional interactions between the nascent tumor and its surrounding stroma [1–3]. In that regard, stromal cells generate oncogenic signals that mediate phenotypic and genomic changes to the adjacent epithelial cells and promote tumor invasion and metastasis [4]. One of the pivotal events that highlight stromal remodeling is the transdifferentiation of stromal cells to myofibroblasts [5]. Myofibroblasts are unique smooth-muscle-like fibroblasts which produce various cytokines and growth factors that modulate epithelial and endothelial proliferation, apoptosis, adhesion and migration.

Cyclooxygenase-2 (COX-2), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) have been conclusively shown to be expressed

[*] P.A. Konstantinopoulos and G.P. Vandoros contributed equally to this work.

[**] Corresponding author: Prof. Athanasios G. Papavassiliou, Department of Biological Chemistry, Medical School, University of Athens, 75, M. Asias Street, GR-11527 Athens, Greece. Tel.: +30 210 7462509; Fax: +30 210 7791207; E-mail: papavas@med.uoa.gr

by epithelial cancer cells and to promote colon cancer growth, metastasis and angiogenesis [6,7]. *Cox-2* promoter contains transcriptional regulatory elements for activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB) transcription factors while *vegf* is a well known AP-1 downstream target gene [8–11]. Although the expression and effects of these factors on colon cancer cells have been extensively studied, their expression and role on the stromal cells (particularly on the stromal myofibroblasts) surrounding colon cancer cells remain elusive. In this study we tested the hypothesis that stromal myofibroblasts surrounding colon adenocarcinomas express COX-2, VEGF and EGF receptor (EGF-R). Furthermore, since the transcription factors AP-1 and NF-κB are known to upregulate transcription of *cox-2* and *vegf* genes we also investigated whether activation of AP-1 and NF-κB occurs in stromal myofibroblasts surrounding colon cancers.

To assess the activation of NF-κB pathway, we evaluated the expression of the NF-κB transcription factor as well as the expression of phosphorylated IκB-α (p-IκB-α). NF-κB is retained to the cytoplasm in an inactive state by binding to an inhibitory IκB-α protein that blocks its nuclear localization sequence (NLS) [12]. Multiple stimuli induce phosphorylation of IκB-α at Ser32 leading to dissociation of NF-κB from IκB-α, unmasking of its NLS and stimulation of its transcriptional activity. In that regard, expression of p-IκB-α correlates with activation of NF-κB pathway. In the case of transcription factor AP-1, we examined the expression of its constituents, c-FOS and phosphorylated c-JUN (p-c-JUN).

2. Materials and methods

2.1. Patient selection

Immunohistochemical methodology was performed on formalin-fixed, paraffin-embedded sections from 40 consecutive patients with colon adenocarcinomas. The age of the patients ranged from 45 to 90 years (mean age: 72 years, SD = 9.8 years). Colon adenocarcinomas were classified as well, moderately and poorly differentiated, according to standard pathologic criteria. Staging was performed according to the American Joint Committee on Cancer (AJCC) system.

2.2. Immunohistochemical methodology

Tissue specimens obtained from tumors were fixed in 10% (v/v) buffered formalin and embedded in paraffin. Serial 5-μm sections were obtained for staining with hematoxylin and eosin and for immunohistochemistry. The antibodies employed in the present study, their sources and their characteristics are depicted in Table 1.

For transcription factors NF-κB and AP-1 (p-c-JUN, c-FOS), nuclear staining suggests that they are in the nucleus and their transcriptional activity is stimulated. For p-IκB-α, cytoplasmic and nuclear staining suggests that IκB-α is phosphorylated and thus inactivated, which leads to translocation of NF-κB to the nucleus and stimulation of its transcriptional activity. For COX-2 and VEGF, cytoplasmic staining indicates that these factors are expressed in the cells. Finally, for EGF-R, membranic staining indicates that EGF-R is expressed in the cells.

Microwave irradiation in 0.01 M citric buffer (pH 6.0) was performed as antigen retrieval method in all cases except for EGF-R. In the case of EGF-R, enzymatic pretreatment with pepsin (DIGEST-ALL, Zymed Laboratories Inc.) was done for 10 min in 37°C. All primary antibodies were diluted with Chem-Mate (DakoCytomation) antibody diluent and applied on tissue sections for 30 min at room temperature.

Immunoreactivity was detected employing the En-Vision (DakoCytomation) protocol in all cases except

Table 1
Sources and characteristics of antibodies used in the present study

Antibody	Company	Type	Immunogen	Dilution
c-FOS	Santa Cruz Biotechnology	Rabbit polyclonal	Highly conserved domain of human c-FOS	1:80
p-c-JUN	Santa Cruz Biotechnology	Mouse monoclonal	Amino acids 59-69 of human c-JUN phosphorylated on Ser63	1:80
EGF-R	Zymed Laboratories Inc.	Mouse monoclonal	Human EGF-R derived from A-431 cells	1:80
p-IκB-α	Santa Cruz Biotechnology	Mouse monoclonal	Amino acid containing phosphorylated Ser32 of IκB-α of human origin	1:80
COX-2	Assay Designs, Inc.	Rabbit polyclonal	Human COX-2	1:60
VEGF	BD Biosciences Pharmingen	Mouse monoclonal	Native human VEGF, clone G153-694	1:100
NF-κB	Santa Cruz Biotechnology	Mouse monoclonal	Amino acids 1-286 of human NF-κB p65	1:100

for EGF-R. EGF-R immunopositivity was detected using the Super Picture Polymer Detection Kit (Zymed Laboratories Inc.). Step sections were stained with all antibodies and a-SMA (a-smooth muscle cell actin – universal marker of myofibroblasts) and comparison of staining results was performed.

2.3. Double immunostaining

To further verify that stromal myofibroblasts were indeed expressing each of the molecular factors we performed double immunostaining with the universal marker for myofibroblasts (a-smooth muscle actin/ a-SMA) as second primary antibody.

Following EnVision protocol and reaction with DAB, slides were incubated with the second primary antibody (a-SMA) for 25 min. Then, incubation with EnVision Alkaline Phosphatase Kit (DakoCytomation) for 25 min followed. Finally, reaction with FAST RED chromogen from the same kit (DakoCytomation) for 20 min was performed. Sections from colon adenocarcinomas, in which the primary antibodies were omitted, were used as negative controls.

2.4. Staining score and statistical analyses

Immunopositivity was graded according to the intensity of the stain i.e. negative (0), weak (+1) and strong (+2). This scale was used by two investigators to evaluate and score all sections independently without prior knowledge of the clinicopathological characteristics of each case. Specimens with interobserver disagreement were reassessed by simultaneous examination by the two pathologists in a double-headed light microscope. The correlation of EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN expression with COX-2 and VEGF expression was assessed by Kendall's t coefficient. The level of expression of all these factors was compared among the three adenocarcinoma differentiation degrees and the four AJCC stages by nonparametric Kruskal–Wallis analysis of variance (ANOVA). All analyses were performed using SPSS 9.0 for Windows.

3. Results

3.1. VEGF, p-IκB-α, NF-κB, c-FOS, p-c-JUN, EGF-R and COX-2 are expressed in stromal myofibroblasts surrounding colon adenocarcinomas

VEGF and COX-2 were expressed in the cytoplasm of stromal myofibroblasts in 80% of the patients and 85% of the cases, respectively. The active form of c-JUN (p-c-JUN) was detected in the nuclei of stromal myofibroblasts in 70% of the patients, while c-FOS was expressed in the cytoplasm and nuclei of stromal myofibroblasts in 85% of the patients. P-IκB-α was expressed in the cytoplasm and nuclei of stromal myofibroblasts in 62.5%, while NF-κB was expressed in the cytoplasm and nuclei of stromal myofibroblasts in 75% of the patients. Finally, membranic staining for EGF-R was present in stromal myofibroblasts in 65% of the cases. The immunohistochemistry results for COX-2, p-IκB-α, NF-κB, EGF-R, VEGF, c-FOS and p-c-JUN are presented in Table 2 and illustrated in Fig. 1. Staining in the cancer cells was also positive and correlated with the staining of the stromal myofibroblasts in all cases.

Table 2
EGF-R, p-IκB-α, NF-κB, c-FOS, p-c-JUN, COX-2 and VEGF expression in the stroma of colon adenocarcinomas

Myofibroblasts	Negative	Weak (+1)	Strong (+2)
COX-2 ($N = 40$)	6 (15%)	11 (27.5%)	23 (57.5%)
p-IκB-α ($N = 40$)	15 (37.5%)	7 (17.5%)	18 (45%)
EGF-R ($N = 40$)	14 (35%)	8 (20%)	18 (45%)
VEGF ($N = 40$)	8 (20%)	12 (30%)	20 (50%)
c-FOS ($N = 40$)	6 (15%)	8 (20%)	26 (65%)
NF-κB ($N = 40$)	14 (35%)	8 (20%)	18 (45%)
p-c-JUN ($N = 40$)	12 (30%)	19 (47.5%)	9 (22.5%)

EGF-R, p-IκB-α, NF-κB, c-FOS, p-c-JUN, COX-2 and VEGF expression was observed in stromal myofibroblasts surrounding colon adenocarcinomas regardless of clinical stage and grade of differentiation. Specifically, EGF-R, p-IκB-α, NF-κB, c-FOS, p-c-JUN, COX-2 and VEGF expression in myofibroblasts did not show any correlation with the staging or differentiation of the adenocarcinomas.

3.2. EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN correlate positively with COX-2 expression in stromal myofibroblasts

The correlation of EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN expression with COX-2 expression was assessed by Kendall's t coefficient. EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN were statistically significantly positively correlated with COX-2 expression ($P < 0.001$, $P = 0.007$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively). These data indicate that there is a coordinated upregulated expression of EGF-R, p-IκB-α, NF-κB, c-FOS, p-c-JUN and COX-2 in stromal myofibroblasts surrounding colon adenocar-

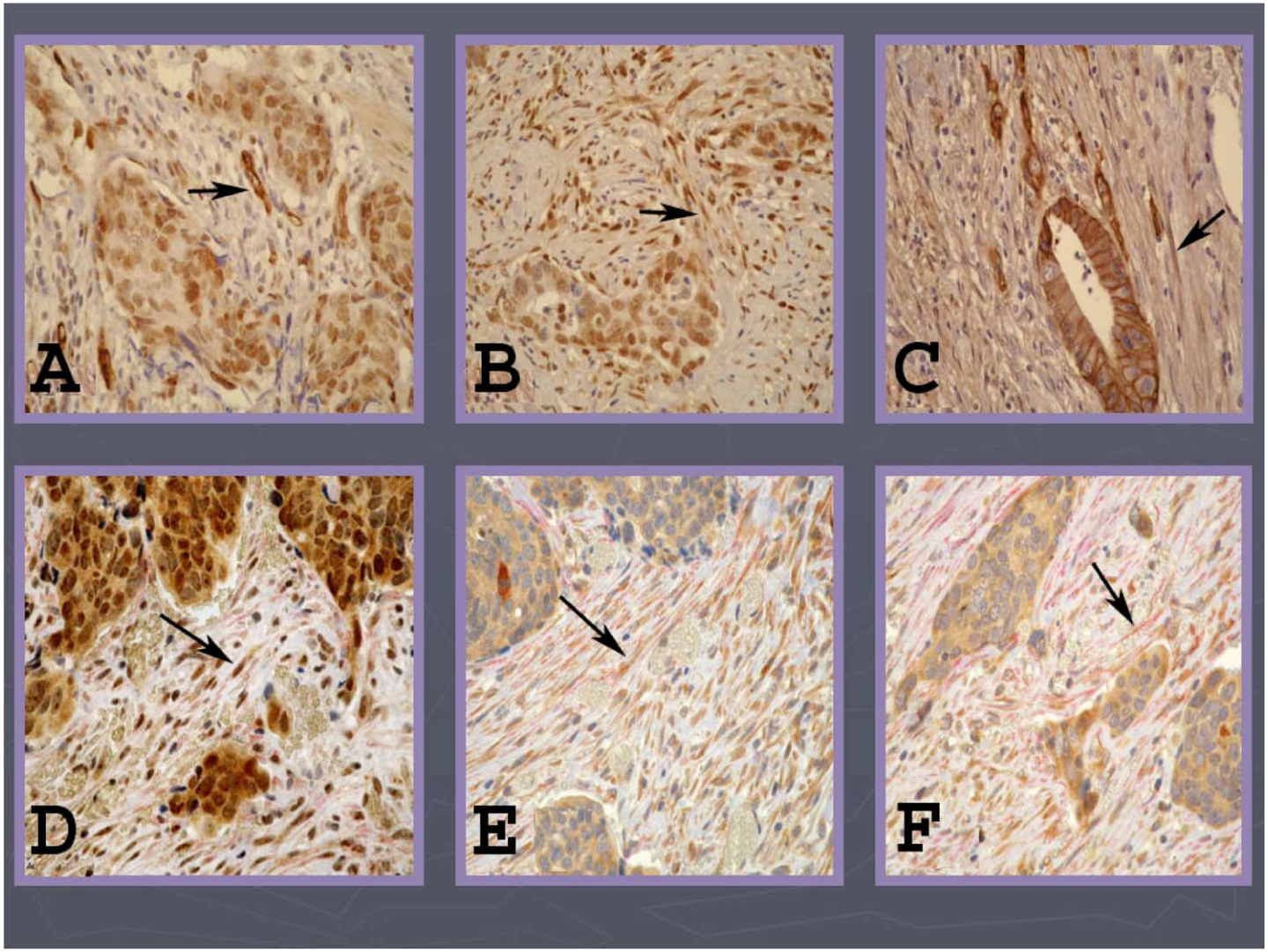

Fig. 1. *Upper Panel*: Myofibroblasts (black arrows) surrounding colon adenocarcinomas exhibiting p-c-JUN (A), c-FOS (B) and EGF-R (C) expression, respectively (×400). P-c-JUN, c-FOS exhibit nuclear staining while EGF-R staining is membranic. *Lower Panel*: Double immunolabeling of stromal myofibroblasts with a-SMA (a-smooth muscle cell actin) and p-IκB-α (D), VEGF (E) and COX-2 (F), respectively (×400). P-IκB-α (D), VEGF (E) and COX-2 (F) staining is brown while a-SMA staining is red in all cases. COX-2 and VEGF exhibit cytoplasmic staining while p-IκB-α is nuclear and cytoplasmic.

cinomas, implying that the activation of EGF-R, AP-1 and NF-κB is associated with increased expression of COX-2 by these cells.

3.3. *EGF-R, p-IκB-α, c-FOS and p-c-JUN correlate positively with VEGF expression in stromal myofibroblasts*

As in the case of COX-2, EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN were statistically significantly positively correlated with VEGF expression (all $P < 0.001$). Kendall's t coefficient was used for this analysis. This result also suggests that coordinated activation of EGF-R, AP-1 and NF-κB is associated with increased expression of VEGF by stromal myofibroblasts surrounding colon adenocarcinomas.

4. Discussion

The present retrospective immunohistochemical study demonstrated that myofibroblasts surrounding colon adenocarcinomas express VEGF, p-IκB-α, NF-κB, c-FOS, p-c-JUN, EGF-R and COX-2. Moreover, EGF-R, p-IκB-α, NF-κB, c-FOS and p-c-JUN were positively correlated with VEGF and COX-2 in a statistically significant manner. Our findings regarding COX-2 are consonant with previous results reported by Shattuck-Brandt et al., Adegboyega et al. and by our group who also detected COX-2 expression in stromal myofibroblasts surrounding colon adenocarcinomas [13–15]. To our knowledge, the present study demonstrates for the first time that stromal myofibroblasts also express VEGF, EGF-R, NF-κB, c-FOS and p-c-JUN regardless of the stage and degree of differentiation of the neoplasm.

Myofibroblasts are unique smooth muscle-like fibroblasts which play a central role in the stromal changes associated with carcinogenesis [5]. In response to cancer cell-derived cytokines such as transforming growth factor-beta (TGF-b), fibroblasts differentiate into myofibroblasts which secrete pro-invasive signals that promote proliferation, mobility and invasion of the adjacent epithelial cells. Expression of COX-2 in myofibroblasts surrounding colon adenocarcinomas indicates that these cells may be responsible for paracrine secretion of prostaglandins (PGs), especially PGE2. PGE2 is a well-known oncogenic signal that promotes cancer cell proliferation and invasiveness and inhibits apoptosis [16]. Furthermore, PGE2 stimulates tumor angiogenesis via direct effects on endothelial cells [16]. This evidence suggests that myofibroblasts may occupy a central role in promoting colon carcinogenesis by exerting significant paracrine influence on adjacent epithelial and endothelial cells to promote tumor invasiveness and angiogenesis, respectively.

Expression of COX-2 in stromal myofibroblasts may be induced by multiple stimuli. COX-2 behaves as an immediate-early gene, which is subject to rapid regulation at the transcriptional level [8–11]. The *cox-2* promoter contains transcriptional regulatory elements for various transcription factors including NF-κB and AP-1. The present study revealed a very strong correlation between activation of NF-κB pathway and COX-2 expression in stromal myofibroblasts, suggesting that NF-κB pathway may constitute a major inducer of *cox-2* gene expression in these cells. Multiple factors can potentiate NF-κB pathway in stromal myofibroblasts. Interleukin-1 (IL-1) can induce *cox-2* gene expression through activation of NF-κB pathway in cultured human intestinal myofibroblasts [17]. Tumor epithelial cell-derived TGF-b as well as exposure to lipopolysaccharide (LPS) can also stimulate activation of NF-κB pathway in target cells [18,19]. Furthermore, luminal bacterial components can activate intestinal myofibroblasts expressing toll-like receptors (TLRs) via induction of NF-κB pathway [20]. Phosphorylation of IκB-α to p-IκB-α is a critical step in the activation of NF-κB pathway and is precisely the point where non-steroidal anti-inflammatory drugs (NSAIDs), which have been used for chemoprevention against colon cancer, act to suppress NF-κB signaling [21].

Our study also demonstrated that in stromal myofibroblasts surrounding colon adenocarcinomas, expression of AP-1 constituents, p-c-JUN and c-FOS, was statistically significantly correlated with expression of COX-2. Importantly, EGF-R expression was also evident and paralleled both c-FOS/p-c-JUN and COX-2 expression. EGF-R activates the membrane-bound GTPase RAS, leading to activation of the extracellular signal-regulated kinase (ERK) and JUN N-terminal kinase (JNK) signaling pathways [6]. Moreover, NF-κB is downstream of EGF-R. Our study supports this orchestrated EGF-R, c-FOS, p-c-JUN and NF-κB activation which is associated with expression of COX-2 in these cells. Besides stromal myofibroblasts, a similar orchestrated EGF-R, c-FOS, p-c-JUN, NF-κB and COX-2 expression has also been shown in colon cancer epithelial cells [22].

Similarly to COX-2, expression of VEGF was identified in stromal myofibroblasts surrounding colon adenocarcinomas in the majority of cases. This novel finding suggests that myofibroblasts may also be directly

involved in promoting tumor angiogenesis via stimulating adjacent endothelial cells. EGF-R, p-IκB-α, c-FOS and p-c-JUN correlated positively with VEGF expression in stromal myofibroblasts, indicating that activation of EGF-R and of downstream NF-κB and AP-1 may be associated with production of VEGF by these cells.

In conclusion, the present study supports a pivotal role of myofibroblasts in the stromal changes associated with colon carcinogenesis. Accordingly, we have demonstrated that these cells are an important source of VEGF and COX-2 production, which are both known to exert powerful angiogenic, proinvasive and growth-promoting effects. This study emphasizes that the role of NF-κB, AP-1, EGF-R, COX-2 and VEGF in colon carcinogenesis is not confined to the tumor epithelial cells, but extends to the stroma, particularly the stromal myofibroblasts that surround colon adenocarcinomas. Our study supports that development of NF-κB- and AP-1-targeted therapies (following the paradigm of anti-EGF-R- and anti-VEGF-targeted therapies) may be an important strategy against colon cancer. Most importantly, our study raises the exciting possibility that stromal myofibroblasts may be themselves important targets against colon carcinogenesis.

References

[1] S.Z. Haslam and T.L. Woodward, Host microenvironment in breast cancer development: epithelial-cell–stromal-cell interactions and steroid hormone action in normal and cancerous mammary gland, *Breast Cancer Res.* **5** (2003), 208–215.

[2] F.T. Bosman, A. de Bruine, C. Flohil, A. van der Wurff, J. ten Kate and W.W. Dinjens, Epithelial–stromal interactions in colon cancer, *Int. J. Dev. Biol.* **37** (1993), 203–211.

[3] L.W. Chung, C.L. Hsieh, A. Law, S.Y. Sung, T.A. Gardner, M. Egawa, S. Matsubara and H.E. Zhau, New targets for therapy in prostate cancer: modulation of stromal–epithelial interactions, *Urology* **62** (2003), 44–54.

[4] T.D. Tlsty and P.W. Hein, Know thy neighbor: stromal cells can contribute oncogenic signals, *Curr. Opin. Genet. Dev.* **11** (2001), 54–59.

[5] D.W. Powell, R.C. Mifflin, J.D. Valentich, S.E. Crowe, J.I. Saada and A.B. West, Myofibroblasts. I. Paracrine cells important in health and disease, *Am. J. Physiol.* **277** (1999), 1–9.

[6] A. Citri and Y. Yarden, EGF-ERBB signalling: towards the systems level, *Nat. Rev. Mol. Cell. Biol.* **7** (2006), 505–516.

[7] R.A. Gupta and R.N. Dubois, Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2, *Nat. Rev. Cancer* **1** (2001), 11–21.

[8] T. Kosaka, A. Miyata, H. Ihara, S. Hara, T. Sugimoto, O. Takeda, E. Takahashi and T. Tanabe, Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2, *Eur. J. Biochem.* **221** (1994), 889–897.

[9] Y.S. Guo, M.R. Hellmich, X.D. Wen and C.M. Townsend, Activator protein-1 transcription factor mediates bombesin-stimulated cyclooxygenase-2 expression in intestinal epithelial cells, *J. Biol. Chem.* **276** (2001), 22941–22947.

[10] R.N. Dubois, S.B. Abramson, L. Crofford, R.A. Gupta, L.S. Simon, L.B. Van De Putte and P.E. Lipsky, Cyclooxygenase in biology and disease, *FASEB J.* **12** (1998), 1063–1073.

[11] C. Michiels, E. Minet, G. Michel, D. Mottet, J.P. Piret and M. Raes, HIF-1 and AP-1 cooperate to increase gene expression in hypoxia: role of MAP kinases, *IUBMB Life* **52** (2001), 49–53.

[12] P.A. Baeuerle and D. Baltimore, NF-kappa B: ten years after, *Cell* **87** (1996), 13–20.

[13] R.L. Shattuck-Brandt, G.W. Varilek, A. Radhika, F. Yang, M.K. Washington and R.N. DuBois, Cyclooxygenase 2 expression is increased in the stroma of colon carcinomas from IL-10(-/-) mice, *Gastroenterology* **118** (2000), 337–345.

[14] P.A. Adegboyega, O. Ololade, J. Saada, R. Mifflin, J.F. Di Mari and D.W. Powell, Subepithelial myofibroblasts express cyclooxygenase-2 in colorectal tubular adenomas, *Clin. Cancer Res.* **10** (2004), 5870–5879.

[15] G.P. Vandoros, P.A. Konstantinopoulos, G. Sotiropoulou-Bonikou, A. Kominea, G.I. Papachristou, M.V. Karamouzis, M. Gkermpesi, I. Varakis and A.G. Papavassiliou, PPARgamma is expressed and NF-κB pathway is activated and correlates positively with COX-2 expression in stromal myofibroblasts surrounding colon adenocarcinomas, *J. Cancer Res. Clin. Oncol.* **132** (2006), 76–84.

[16] M. Majima, H. Amano and I. Hayashi, Prostanoid receptor signaling relevant to tumor growth and angiogenesis, *Trends Pharmacol. Sci.* **24** (2003), 524–529.

[17] R.C. Mifflin, J.I. Saada, J.F. Di Mari, P.A. Adegboyega, J.D. Valentich and D.W. Powell, Regulation of COX-2 expression in human intestinal myofibroblasts: mechanisms of IL-1-mediated induction, *Am. J. Physiol. Cell Physiol.* **282** (2002), 824–834.

[18] M. Kojima, T. Morisaki, K. Izuhara, A. Uchiyama, Y. Matsunari, M. Katano and M. Tanaka, Lipopolysaccharide increases cyclo-oxygenase-2 expression in a colon carcinoma cell line through nuclear factor-kappa B activation, *Oncogene* **19** (2000), 1225–1231.

[19] T. Lu, L.G. Burdelya, S.M. Swiatkowski, A.D. Boiko, P.H. Howe, G.R. Stark and A.V. Gudkov, Secreted transforming growth factor beta2 activates NF-kappaB, blocks apoptosis, and is essential for the survival of some tumor cells, *Proc. Natl. Acad. Sci. USA* **101** (2004), 7112–7117.

[20] J.M. Otte, I.M. Rosenberg and D.K. Podolsky, Intestinal myofibroblasts in innate immune responses of the intestine, *Gastroenterology* **124** (2003), 1866–1878.

[21] M.J. Yin, Y. Yamamoto and R.B. Gaynor, The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta, *Nature* **396** (1998), 77–80.

[22] P.A. Konstantinopoulos, G.P. Vandoros, G. Sotiropoulou-Bonikou, A. Kominea and A.G. Papavassiliou, NF-kappaB/PPARgamma and/or AP-1/PPARgamma 'on/off' switches and induction of CBP in colon adenocarcinomas: correlation with COX-2 expression, *Int. J. Colorectal Dis.* **22** (2007), 57–68.