Exp Oncol 2005
27, 2, 108–113

Experimental ONCOLOGY

PGE_2 UP–REGULATES VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN MKN28 GASTRIC CANCER CELLS VIA EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING SYSTEM

Yan-Bing Ding[1,], Rui-Hua Shi[2,**], Jian-Dong Tong[1], Xiao-Yu Li[3], Guo-Xin Zhang[2], Wei-Ming Xiao[1], Jian-Guo Yang[1], Yun Bao[1], Jian Wu[1], Zhi-Gang Yan[1], Xue-Hao Wang[4,**]*

[1]*Department of Gastroenterology and Oncology, Yangzhou First Municipal Hospital, Yangzhou University, Yangzhou 225001, Jiangsu Province, China*

[2]*Department of Gastroenterology, the First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China*

[3]*Department of Atherosclerosis Research Center, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China*

[4]*Liver Transplantation Center, the First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China*

Aim: **1) To evaluate the effect of prostaglandin E_2 (PGE_2) on the regulation of vascular endothelial growth factor (VEGF) expression in gastric MKN28 cells, and 2) to investigate the role of the epidermal growth factor receptor (EGFR) signal transduction pathway in any effect exerted by PGE_2 on VEGF expression.** ***Methods:*** **MKN28 cells were incubated with the vehicle (control) or with PGE_2 in the presence or absence of AG1478, a selective inhibitor of EGFR tyrosine kinase, or PD098059, a selective inhibitor of the kinase responsible for ERK2 phosphorylation (mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK)). Real-time quantitative polymerase chain reaction and Western blot analysis were used to evaluate VEGF mRNA and protein expression. The activity of EGFR and ERK2 was measured by Western blot analysis.** ***Results:*** **PGE_2 significantly up-regulated VEGF mRNA and protein expression and increased the activation of EGFR and ERK2. Incubation of MKN28 cells with AG1478 significantly reduced PGE_2-induced EGFR activity, ERK2 activity, and VEGF mRNA and protein expression. Meanwhile, incubation of MKN28 with PD098059 reduced PGE_2-induced ERK2 activity and VEGF mRNA and protein expression, but had no effect on EGFR activity.** ***Conclusion:*** **Our data suggested that PGE_2 up-regulates VEGF expression in gastric cancer cells via transactivation of EGFR-MAPK signaling pathways, which may be mechanisms underlying the contribution of COX-2 to tumor angiogenesis in gastric cancer.**

Key Words: **COX-2, PGE_2, gastric cancer, VEGF, EGFR, ERK2, MAPK.**

It is now clear that COX–2 plays a role in the development of gastric and colorectal cancer [1–3]. Recent studies have demonstrated that COX–2 could affect carcinogenesis *via* several different mechanisms. COX–2–mediated PG biosynthesis has been suggested to be involved in the development of cancer based on elevated levels of PGs, especially PGE_2, in cancer tissues [4–6]. However, the precise contribution of increased biosynthesis of PGE_2 to the progression of gastric cancer is not completely understood.

Vascular endothelial growth factor (VEGF), the most well–characterized angiogenic factor [7], plays a critical role in tumor–associated microvascular angiogenesis [8] and is overexpressed in human gastric carcinomas [9–11]. Recent studies have shown that there is a significant correlation between COX–2 and VEGF expression in human gastric cancer, indicating that prostaglandins may possibly play an important role in gastric cancer angiogenesis [12, 13]. Also, Pai et al. [14] and Cheng et al. [15] have demonstrated that PGE_2 stimulates VEGF expression in cultured rat endothelial cells and rat Muller cells. It is not known whether PGE_2 can regulate VEGF expression in human gastric cancer cells. Also, the mechanism by which COX–2–generated PGE_2 induces VEGF expression has not been fully elucidated. Recently Pai et al. [16] demonstrated that PGE_2 can transactivate epidermal factor growth receptor (EGFR) and trigger mitogenic signaling in cultured gastric epithelial and colon cancer cells as well as in rat gastric mucosa *in vivo*, presenting a novel mechanism for promoting colon cancer growth *via* gastrointestinal hypertrophy. We hypothesized that the effects of PGE_2 on VEGF are mediated by EGFR activation. Therefore, this study was designed first to evaluate whether PGE_2 alters VEGF expression in gastric cancer cells *in vitro*. Second, we examined whether the EGFR signal transduction pathway is involved in any effects exerted by PGE_2 on VEGF expression. We found that PGE_2 up–regulates VEGF expression in gastric cancer cells and that this effect is mediated by the EGFR activation.

MATERIALS AND METHODS

Materials. Antibodies to EGFR, ERK2, VEGF, and β–Actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to phospho–EGFR (phospho–EGFR Tyr1173) and to phospho–extracellular sig–

Received: April 25, 2005.
*Correspondence: Fax: 86–0514–7213549
E–mail: chinadyb@hotmail.com
**Co–correspondence.

nal–regulated kinase (ERK2) (Phospho–p44/42 MAPK Thr202/Tyr204) were purchased from Upstate Biotechnology (Lake Placid, NY) and from Cell Signaling Technology respectively. PGE_2, PD098059 and AG1478 were purchased from Calbiochem (La Jolla, CA).

Cell culture. The MKN28 cell line was a generous gift from Professor Yunling Wu (Department of Gastroenterology, RuiJin Hospital, Shanghai). This cell line derives from a well–differentiated human gastric tubular adenocarcinoma and shows gastric–type differentiation [17]. MKN28 cells were routinely grown as monolayers at 37 °C in a humidified atmosphere of 5% CO_2 in Dulbecco's Modified Eagle Medium: Nutrient Mixture F–12 (D–MEM/F–12) 1 : 1 Mixture (GIBCO® Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal calf serum (Life Technologies, Inc).

Western blot analysis. Cells were washed with ice–cold PBS and broken open in lysis buffer. The protein content of the clarified lysate was determined using a BCA protein assay kit (Pierce Chemical Co., Rockford, IL). Cell lysates containing equal amounts of proteins were subjected to SDS–PAGE and transferred onto a nitrocellulose membrane. Blots were stained with Ponceau Red to ensure equal loading and complete transfer of proteins. The blot was incubated with a blocking buffer, and probed with specific primary antibodies (listed under Materials). Blots were then washed and incubated with specific peroxidase–conjugated secondary antibodies. After washing, bound antibody was visualized by an ECL detection system (Amersham Corp., Arlington Heights, IL) according to the manufacturer's instructions. The density of the protein bands was analyzed using a video image analysis system (Image–1/FL, Universal Imaging Corp., Westchester, PA).

RNA extraction. Total RNA was isolated from MKN28 cells using an RNA extraction reagent, TRIzol® (Invitrogen Life Technologies, Inc., Grand Island, NY), according to the standard acid–guanidium–phenol–chloroform method.

Real–time quantitative polymerase chain reaction. Quantitative real–time PCR was performed with the LightCycler thermal cycling system (Roche Diagnostics, Indianapolis, IN) using the RNA Master SYBR Green I kit (Roche Diagnostics) as described by the manufacturer. PCR was carried out in 20 μl of LightCycler RNA Master SYBR Green I solution containing 3.25 mM manganese acetate and a 0.3 μM concentration of each primer. Reverse transcription was carried out at 61 °C for 20 min. Subsequent PCR amplification was initiated with an incubation at 95 °C for 2 min, followed by 45 cycles of 95 °C for 5 s, 50 °C for 5 s, and 72 °C for 20 s. VEGF primers sequences were 5′–CCTGGTGGACATCTTCCAGGAGTACC–3′ (sense) and 5′–GAAGCTCATCTCTCCTATGTGCTGGC–3′ (antisense). The primers for GAPDH were 5′–CACCATCTTCCAGGAGCGAG–3′ (sense) and 5′–TCACGCCACAGTTTCCCGGA–3′ (antisense). Primers were synthesized by Shanghai Sangon Co., Ltd. Data analysis was done using the Roche Molecular Biochemicals LightCycler software (version 3.3). The specificity of the amplification reactions was confirmed by analyzing their corresponding melting curves.

Statistical analysis. All data are reported as the mean ± SD. The statistical significance of differences between mean values was assessed by the Student's *t*–test for unpaired data. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of PGE_2 on VEGF mRNA expression. MKN28 cells were treated with varying concentrations (0.1 μM–10 μM) of PGE_2. Exposure of MKN28 cells to PGE_2 for 3 h caused a dose–dependent increase in VEGF mRNA expression (Fig. 1, *a, b*).

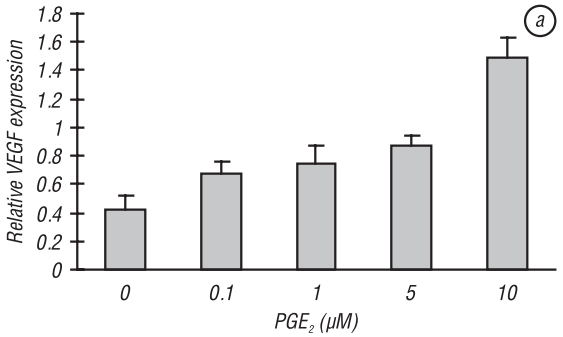

Fig. 1, *a.* Dose–dependent response of VEGF mRNA expression to Prostaglandin E_2 concentration
Serum–starved MKN28 cells were treated with varying concentrations of PGE_2 (0.1 μM to 10 μM) for 3 h. Messenger RNA was isolated and used as a template for a real–time quantitative reverse transcription–polymerase chain reaction assay to measure the relative levels of VEGF as described under Materials and Methods. Values are the mean intensity ± SD of three separate experiments

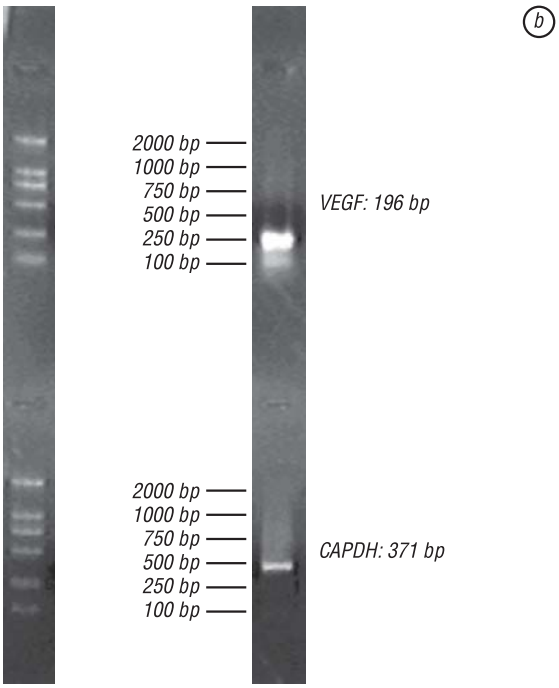

Fig. 1, *b.* Analysis of the real time PCR products on the 1.5% agarose gel electrophoresis
The real time PCR products were analysed on the 1.5% agarose gel electrophoresis. The results showed that the length of VEGF was between 100 b and 250 bp, and the length of GAPDH was between 250 b and 500 bp. They were consistent with the length of designed fragment

Effect of PGE_2 on activation of EGFR and ERK2 kinase. Because PGE_2 has been shown to transactivate EGFR and stimulate the downstream ERK2 signaling pathway in colon cancer cells [16], we investigated whether the PGE_2–mediated increase in VEGF expression involves cross–communication between PGE_2 and the EGFR signaling systems. Treatment of MKN28 cells with PGE_2 (0.1 μM to 10 μM, 5 min) significantly increased EGFR and ERK2 phosphorylation (Fig. 2, 3) in a dose–dependent manner. To further ascertain the role of PGE_2–induced EGFR activation in triggering the mitogenic pathway, we abolished EGFR and ERK2 kinase activity with specific inhibitors and measured PGE_2–induced EGFR and ERK2 activity. MKN28 cells were pretreated with either AG1478 (an EGFR kinase inhibitor, 250 nM) for 20 min or PD98059 (a MEK inhibitor, 20 μM) for 50 min, followed by PGE_2 (10 μM, 5 min) treatment. AG1478 almost completely abolished PGE_2–induced EGFR and ERK2 activation without altering their protein levels (Fig. 4, *a*, *b*). PD98059 treatment significantly inhibited PGE_2–induced ERK2 activity but had no effect on EGFR activation (Fig. 4, *a*, *b*). These results clearly indicated that PGE_2–induced ERK2 activation is mediated via EGFR.

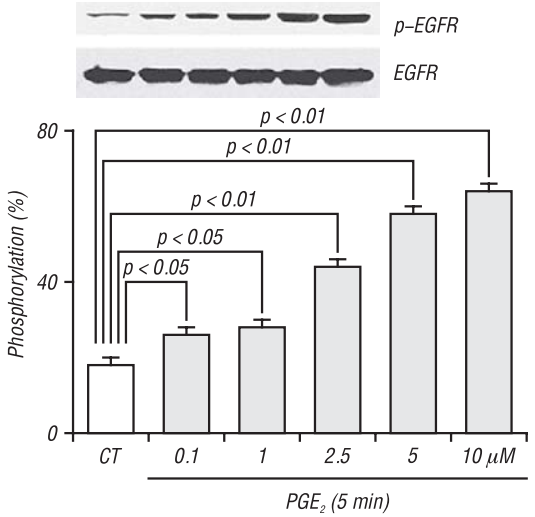

Fig. 2. PGE_2 dose–dependently increases EGFR activity in MKN28 cells
Serum–starved MKN28 cells were treated with varying concentrations of PGE_2 (0.1 μM to 10 μM) for 5 min, after which total cellular protein was collected. EGFR was immunoprecipitated, and phosphorylation (p–EGFR) was visualized with anti– phospho–EGFR antibodies (*top*). The total amount of EGFR in immunoprecipitates was determined by Western blot analysis with antibodies to EGFR (*middle*). Quantitative analysis of EGFR phosphorylation by determining the ratio between EGFR and p–EGFR (*mean ± SD*) from 3 separate experiments each performed in triplicate (*bottom*)

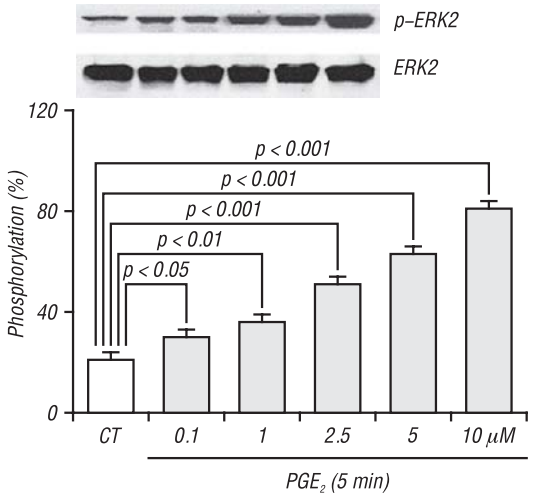

Fig. 3. PGE_2 dose–dependently increases ERK2 activity in MKN28 cells
Serum–starved MKN28 cells were treated with varying concentrations of PGE_2 (0.1 μM to 10 μM) for 5 min, after which total cellular protein was collected. ERK2 was immunoprecipitated, and phosphorylation(p–ERK2) was visualized with anti– phospho–ERK2 antibodies (*top*). The total amount of ERK2 in immunoprecipitates was determined by Western blot analysis with antibodies to ERK2 (*middle*). Quantitative analysis of ERK2 activity (*mean ± SD*) from 3 separate experiments each performed in triplicate (*bottom*)

Effect of AG1478 and PD98059 on VEGF mRNA expression. Because PGE_2 significantly increased both VEGF mRNA expression and the activity of EGFR signaling systems, we examined whether PGE_2–induced VEGF mRNA expression involves the EGFR and the ERK2 pathway. Pretreatment of MKN28 cells with a MEK inhibitor (PD98059, 20 μM for 50 min) or an EGFR inhibitor (AG1478, 250 nM for 20 min) significantly reduced PGE_2–induced VEGF mRNA (Fig. 5), indicating involvement of both EGFR and ERK2 in PGE_2–mediated upregulation of VEGF mRNA expression.

Effect of PGE_2 on VEGF protein expression. To ascertain whether PGE_2–induced VEGF mRNA expression accompanies an increase in VEGF protein expression, MKN28 cells were treated with PGE_2 (10 μM) for 3, 6 and 24 h. VEGF protein expression was significantly increased at all of the time points studied, with a maximal response at 3 h. Pre–treatment of MKN28 cells with PD98059 or AG1478 significantly reduced the PGE_2–induced increase of VEGF protein expression (Fig. 6), suggesting involvement of the EGFR and ERK2 pathways.

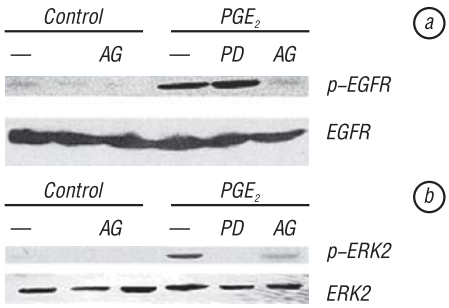

Fig. 4. PGE_2 induced activation of ERK2 involves EGFR
a — MKN28 cells were serum starved for 48 h, and treated with 20 μM PD98059 (50 min prior to PGE_2 treatment), AG1478 250 nM (20 min prior to PGE_2 treatment) or DMSO (control; no PGE_2 treatment). After PGE_2 (10 μM) stimulation for 5 min, total cellular protein was collected. Equal amounts of protein were separated by SDS–PAGE and phosphorylated EGFR(p–EGFR) was visualized with anti–phospho– EGFR antibodies. Equal loading of lysates is shown by Western blot analysis with antibodies to EGFR; *b* — MKN28 cells were serum starved for 48 h, and treated with 20 μM PD98059 (50 min prior to PGE_2 treatment), AG1478 250nM (20 min prior to PGE_2 treatment) or DMSO (control). After PGE_2 (10 μM) stimulation for 5 min, total cellular protein was collected. Equal amounts of protein were separated by SDS–PAGE and phosphorylated ERK2(p–ERK2) was visualized with anti– phospho–ERK2 antibodies. Equal loading of lysates is shown by Western blot analysis with antibodies to ERK2

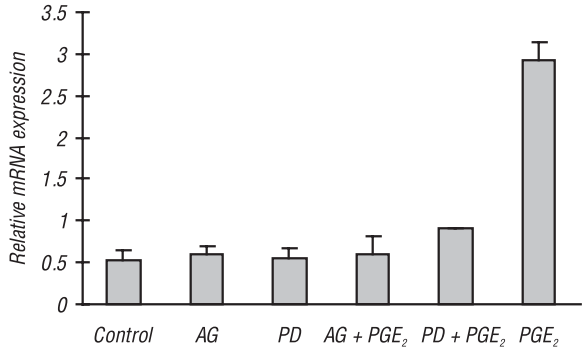

Fig. 5. Inhibition of EGFR or ERK2 activation reduces PGE_2–induced VEGF mRNA expression in MKN28 cells
MKN28 cells were treated for 3 h with 10 μM PGE_2. Messenger RNA was isolated and used as a template for a real–time quantitative reverse transcription– polymerase chain reaction assay to measure the relative levels of VEGF. Values are the mean intensity ± SD of three separate experiments

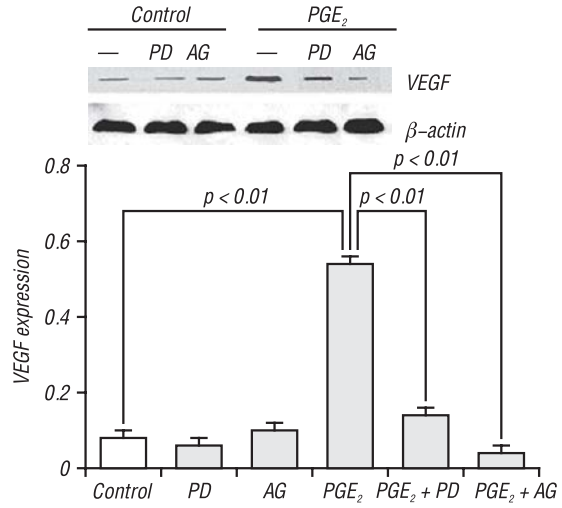

Fig. 6. PGE_2 induces VEGF protein expression in MKN28 cells
Serum–starved MKN28 cells were treated with either vehicle, PD98059 (20 μM, 50 min), AG1478 (250 nM, 20 min), PGE_2 (10 μM, 3 h), PD plus PGE_2, or AG plus PGE_2. Detergent–solubilized cell lysates (150 mg protein per lane) were subjected to Western blot analysis using a specific polyclonal antibody against VEGF (*upper*). Values are the mean relative density ± SD of three separate experiments (*bottom*)

DISCUSSION

It is now clear that COX–2 plays a role in the promotion of gastric cancer [18]. In addition, VEGF has been found to be overexpressed in gastric cancer [9–11]. Recent studies have shown that the COX–2 expression is significantly correlated with VEGF expression in human gastric cancer [12, 13], and that COX–2 metabolic products contribute to neovascularization and support vasculature–dependent solid tumor growth and metastasis [19–21]. A few reports have also indicated that PGE_2, generated by COX–2, can up–regulate VEGF in cultured rat endothelial cells and rat Muller cells [14, 15]. However, the relationship between PGE_2 and VEGF expression in human gastric cancer has not been studied extensively. Here we provide evidence that PGE_2 can up–regulate VEGF in human gastric cancer cells. Recently, Leung et al. [22] reported that forced expression of COX–2 in gastric cancer cells results in increased VEGF expression, strongly consistent with our results, indicating that PGE_2 stimulates VEGF expression in gastric cancer cells. These findings may explain, at least in part, the observed phenomenon that COX–2 over–expression correlates with vascular endothelial growth factor expression and tumor angiogenesis in gastric cancer.

The present study also demonstrates for the first time that PGE_2–induced VEGF expression in gastric cancer cells is *via* a PGE_2/EGFR/ERK2 pathway and that EGFR transactivated by PGE_2 plays a critical role in the PGE_2–induced effects. Our finding is in agreement with a recent report showing that *Helicobacter pylori* up–regulates VEGF expression in gastric cancer cells through activation of EGFR and the mitogen–activated protein (MAP) kinase cascade [23]. Meanwhile, Buchanan et al. [24] reported that the early effects of COX–2 derived PGE_2 in developing colonic carcinomas were in part mediated by EGFR. This transactivation was responsible for subsequent downstream effects including the stimulation of cell migration and invasion, and is again indicative of the important role of EGFR in COX–2 related carcinogenesis.

Prostaglandins exert their biological actions via specific cell–surface receptors (EP1,–2,–3,–4) [25]. These receptors are G protein–coupled receptors (GPCR), with seven transmembrane domains. Diverse signaling pathways have been implicated in GPCR mediated transactivation of EGFR [26, 27]. Recently, Pai et al. [16] reported that PGE_2–mediated transactivation of EGFR and downstream signaling in colon cancer cells involves TGF–α, an EGFR ligand, which is likely released by the c–Src–activated matrix metalloproteinase pathway. More recently, Shao et al. [28] demonstrated that PGE_2 transactivated EGFR through the induction of amphiregulin (AR) expression in colon cancer cells. Taken together, these data indicate that PGE_2–mediated transactivation of EGFR in colon cancer cells may be through different mechanisms that possibly act in a synergistic manner. Although a recent report has shown that several gastric cancer cell lines express all four EP receptors (EP1,–2,–3,–4) [29], the exact mechanisms by which the GPCR transactivates EGFR signaling pathways in gastric cancer have so far not been identified. Future studies should be aimed at examining the transcriptional regulation of gene expression through the PGE_2/GPCR/EGFR pathway and the synergistic cooperation among the multiple oncogenic pathways involved in the PGE_2–induced effects. Such findings will be critical for the understanding of COX–2 proneoplastic activity in gastric carcinogenesis.

In conclusion, this study demonstrated for the first time that PGE_2–induced VEGF expression in gastric cancer cells is mediated via EGFR transactivation. These findings provide insight into the mechanism by which COX–2/PGE_2 promotes gastric carcinogenesis. Recently, Torrance et al. [30] showed that the use of a non–selective COX inhibitor in combination with an EGFR inhibitor reduced polyp formation in $APC^{Min/+}$ mice much more effectively than either agent alone. In light of these studies, our findings that PGE_2–induced trans–

activation of the EGFR is required for stimulation of VEGF expression in MKN28 cells are quite intriguing. One may therefore envision the combined use of COX–2 and EGFR inhibitors for gastric cancer prevention or treatment. Therefore experimental and clinical studies that would further evaluate the combination of agents that target both the COX–2 and EGFR pathways should be pursued.

REFERENCES

1. **Uefuji K, Ichikura T, Mochizuki H, Shinomiya N.** Expression of cyclooxygenase-2 protein in gastric adenocarcinoma. J Surg Oncol 1998; **69**: 168–72.

2. **Ristimaki A, Honkanen N, Jankala H, Sipponen P, Harkonen M.** Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 1997; **57**: 1276–80.

3. **Yu LL, Yu HG, Yu JP, Luo HS.** Nuclear factor-kappa B regulates cyclooxygenase-2 expression and cell proliferation in human colorectal carcinoma tissue. Exp Oncol 2004; **26**: 40–7.

4. **Rigas B, Goldman IS, Levine L.** Altered eicosanoid levels in human colon cancer. J Lab Clin Med 1993; **122**: 518–23.

5. **Bennett A, Civier A, Hensby CN, Melhuish PB, Stamford IF.** Measurement of arachidonate and its metabolites extracted from human normal and malignant gastrointestinal tissues. Gut 1987; **28**: 315–8.

6. **McLemore TL, Hubbard WC, Litterst CL, Liu MC, Miller S, McMahon NA, Eggleston JC, Boyd MR.** Profiles of prostaglandin biosynthesis in normal lung and tumor tissue from lung cancer patients. Cancer Res 1998; **58**: 3140–7.

7. **Papetti M, Herman IM.** Mechanisms of normal and tumor-derived angiogenesis. Am J Physiol Cell Physiol 2002; **282**: C947–70.

8. **Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgruber RM, Feder J.** Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989; **84**: 1470–8.

9. **Tian X, Song S, Wu J, Meng L, Dong Z, Shou C.** Vascular endothelial growth factor: acting as an autocrine growth factor for human gastric adenocarcinoma cell MGC803. Biochem Biophys Res Commun 2001; **286**: 505–12.

10. **Amioka T, Kitadai Y, Tanaka S, Haruma K, Yoshihara M, Yasui W, Chayama K.** Vascular endothelial growth factor-C expression predicts lymph node metastasis of human gastric carcinomas invading the submucosa. Eur J Cancer 2002; **38**: 1413–9.

11. **Yamamoto S, Yasui W, Kitadai Y, Yokozaki H, Haruma K, Kajiyama G, Tahara E.** Expression of vascular endothelial growth factor in human gastric carcinomas. Pathol Int 1998; **48**: 499–506.

12. **Yu HG, Li JY, Yang YN, Luo HS, Yu JP, Meier JJ, Schrader H, Bastian A, Schmidt WE, Schmitz F.** Increased abundance of cyclooxygenase-2 correlates with vascular endothelial growth factor-A abundance and tumor angiogenesis in gastric cancer. Cancer Lett 2003; **195**: 43–51.

13. **Joo YE, Rew JS, Seo YH, Choi SK, Kim YJ, Park CS, Kim SJ.** Cyclooxygenase-2 overexpression correlates with vascular endothelial growth factor expression and tumor angiogenesis in gastric cancer. J Clin Gastroenterol 2003; **37**: 28–33.

14. **Pai R, Szabo IL, Soreghan BA, Atay S, Kawanaka H, Tarnawski AS.** PGE_2 stimulates VEGF expression in endothelial cells via ERK2/JNK1 signaling pathways. Biochem Biophys Res Commun. 2001; **286**: 923–8.

15. **Cheng T, Cao W, Wen R, Steinberg RH, LaVail MM.** Prostaglandin E_2 induces vascular endothelial growth factor and basic fibroblast growth factor mRNA expression in cultured rat Muller cells. Invest Ophthalmol Vis Sci 1998; **39**: 581–91.

16. **Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS.** Prostaglandin E_2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002; **8**: 289–93.

17. **Romano M, Razandi M, Sekhon S, Krause WJ, Ivey KJ.** Human cell line for study of damage to gastric epithelial cells *in vitro*. J Lab Clin Med 1988; **111**: 430–40.

18. **Saukkonen K, Rintahaka J, Sivula A, Buskens CJ, Van Rees BP, Rio MC, Haglund C, Van Lanschot JJ, Offerhaus GJ, Ristimaki A.** Cyclooxygenase-2 and gastric carcinogenesis. APMIS 2003; **111**: 915–25.

19. **Uefuji K, Ichikura T, Mochizuki H.** Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. Clin Cancer Res 2000; **6**: 135–8.

20. **Tsujii M, DuBois RN.** Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995; **83**: 493–501.

21. **Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN.** Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998; **93**: 705–16.

22. **Leung WK, To KF, Go MY, Chan KK, Chan FK, Ng EK, Chung SC, Sung JJ.** Cyclooxygenase-2 upregulates vascular endothelial growth factor expression and angiogenesis in human gastric carcinoma. Int J Oncol 2003; **23**: 1317–22.

23. **Caputo R, Tuccillo C, Manzo BA, Zarrilli R, Tortora G, Blanco Cdel V, Ricci V, Ciardiello F, Romano M.** *Helicobacter pylori* VacA toxin up-regulates vascular endothelial growth factor expression in MKN 28 gastric cells through an epidermal growth factor receptor-, cyclooxygenase-2-dependent mechanism. Clin Cancer Res 2003; **9**: 2015–21.

24. **Buchanan FG, Wang D, Bargiacchi F, DuBois RN.** Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem 2003; **278**: 35451–7.

25. **Narumiya S, Sugimoto Y, Ushikubi F.** Prostanoid receptors: structures, properties, and functions. Physiol Rev 1999; **79**: 1193–226.

26. **Hackel PO, Zwick E, Prenzel N, Ullrich A.** Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Curr Opin Cell Biol 1999; **11**: 184–9.

27. **Luttrell LM, Daaka Y, Lefkowitz RJ.** Regulation of tyrosine kinase cascades by G-protein-coupled receptors. Curr Opin Cell Biol 1999; **11**: 177–83.

28. **Shao J, Lee SB, Guo H, Evers BM, Sheng H.** Prostaglandin E2 stimulates the growth of colon cancer cells via induction of amphiregulin. Cancer Res 2003; **63**: 5218–23.

29. **Okuyama T, Ishihara S, Sato H, Rumi MA, Kawashima K, Miyaoka Y, Suetsugu H, Kazumori H, Cava CF, Kadowaki Y, Fukuda R, Kinoshita Y.** Activation of prostaglandin E2-receptor EP2 and EP4 pathways induces growth inhibition in human gastric carcinoma cell lines. J Lab Clin Med 2002; **140**: 92–102.

30. **Torrance CJ, Jackson PE, Montgomery E, Kinzler KW, Vogelstein B, Wissner A, Nunes M, Frost P, Discafani CM.** Combinatorial chemoprevention of intestinal neoplasia. Nat Med 2000; **6**: 1024–8.

РЕГУЛЯЦИЯ ЭКСПРЕССИИ ФАКТОРА РОСТА ЭНДОТЕЛИАЛЬНЫХ КЛЕТОК В КЛЕТКАХ РАКА ЖЕЛУДКА ЧЕЛОВЕКА ЛИНИИ MKN28 С УЧАСТИЕМ ПРОСТАГЛАНДИНА E_2 И СИГНАЛЬНОЙ СИСТЕМЫ РЕЦЕПТОРА ЭФР

Цель: **1) оценить влияние простагландина E_2 (PGE_2) на регуляцию экспрессии VEGF в клетках рака желудка человека линии MKN28, 2) изучить роль сигнального каскада с участием рецептора ЭФР (EGFR) в опосредовании влияния PGE_2 на экспрессию VEGF.** ***Методы:*** **клетки MKN28 инкубировали с PGE_2 или без него (группа контроля) в присутствии или в отсутствие селективного ингибитора тирозин-киназы EGFR AG1478 или селективного ингибитора киназы, ответственной за фосфорилирование ERK2, PD098059. Для оценки уровня экспрессии VEGF на уровне мРНК и белка использовали методы Rt-PCR и Вестерн-блоттинга соответственно. Активность EGFR и ERK2 также определяли методом Вестерн-блоттинга.** ***Результаты:*** **инкубация клеток с PGE_2 приводила к значительному повышению экспрессии VEGF на уровне мРНК и белка, а также активности EGFR и ERK2. Инкубация клеток с AG1478 оказывала ингибирующий эффект на PGE_2-индуцированную активность EGFR и ERK2, равно как и на экспрессию VEGF, в то время как PD098059 не оказывал влияния на активность EGFR, но снижал PGE_2-индуцированную активность ERK2 и экспрессию VEGF.** ***Выводы:*** **наши данные показывают, что PGE_2 усиливает экспрессию VEGF в опухолевых клетках желудка человека путем трансактивации сигнальных каскадов EGFR-MAPK, что может послужить объяснением механизма участия COX-2 в ангиогенезе опухолей желудка.**

Ключевые слова: **COX-2, PGE_2, рак желудка, VEGF, EGFR, ERK2, MAPK.**