J. Tokyo Med. Univ., **60**(3) : 181～188, 2002

Differential effects of cyclooxygenase-2 on vascular endothelial growth factor production in gastric fibroblasts and cancer cells

Shuhei MIURA[1], Taku TSUKUI[2], Kei SHINOKI[2], Hiroki TAKEYAMA[1], Tomonori AKAMATSU[2], Kazumasa MIYAKE[2], Ken WADA[2], Yuji MIZOKAMI[1], Masafumi KOBAYASHI[2], Takeshi MATSUOKA[1] and Choitsu SAKAMOTO[2]

[1]Fifth Department of Internal Medicine, Tokyo Medical University
[2]Third Department of Internal Medicine, Nippon Medical School

Abstract

Details of the regulation of vascular endothelial growth factor (VEGF) production in gastric cancer cells are unclear. To investigate whether VEGF production is involved in cyclooxygenase (COX)-2 expression in gastric cancer cells, we investigated the effects of indomethacin and the selective COX-2 inhibitor, NS-398, on VEGF production in gastric cancer cells and compared the results with those in gastric fibroblasts. We discovered that two kinds of primary cultured gastric fibroblasts could produce VEGF in the media supplemented only with bovine bovine serum (FBS), and the production was inhibited by indomethacin or NS-398. On the contrary, though the gastric cancer cell lines : MKN1, MKN28, MKN74, JR-1, TMK-1, NUGC-3 and AZ-521 expressed VEGF and COX2 mRNA, and also produced VEGF when they were cultured in media supplemented with 10% FBS, indomethacin or NS-398 did not inhibit the production of VEGF in these cells. We also found that the high concentration of NS-398 〔100 μM〕 significantly up-regulated VEGF production of MKN1 and MKN74 even in serum-free media. These results suggested that VEGF production of gastric cancer cells might be up-regulated by the independent pathway of COX-2 expression.

Introduction

Angiogenesis is known to be very important in solid tumor-growth in vivo[1]. Recent papers showed many growth factors, including fibroblast growth factors (FGF), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), are involved in angiogenesis[2]. Another paper also demonstrated that cyclooxygenase (COX)-2 enhanced the production of angiogenic factors in a few colon cancer cells in vitro, and a recent paper also showed a correlation between COX-2 expression and tumor angiogenesis in human colorectal cancer with immunohistochemical analysis[3,4]. However it is not yet clear which kind of mechanism exists between COX-2 expression and the production of angiogenic factors in colon cancer cells. Furthermore, though cyclooxygenase (COX)-2 was reported to be expressed by a variety of tumor cells such as gastric cancer cells, breast cancers, and lung cancers, the direct involvement of COX-2 expression in angiogenic factor- production in these cells is not yet clear[5].

Although possible contributions of COX-2 in tumor angiogenesis include not only the increased expression of angiogenic factors, but also the direct stimulation of

Received December 27, 2001, Accepted February 20, 2002
Key words : Cyclooxygenase-2, Gastric fibroblasts, Gastric cancer cells, Vascular Endothelial Growth Factor
Reprint requests to : Shuhei Miura, Fifth Department of Internal Medicine, Tokyo Medical University 3-20-1 Chuo Ami-machi Inashiki-Gun Ibaraki-ken 300-0385. Tel : 0298-87-1161 ext1128. Fax : 0298-88-3463

endothelial cell-migration and growth by the eicosanoid products, we focused on the effects of COX-2 on VEGF production in gastric cancer cells, because VEGF is one of the most potent and specific growth factors for endothelial cells[6,7,8]. We have already shown that the expression of COX-2 correlates with that of VEGF in human gastric cancer tissue on the basis of immunohistological analysis[9].

In this study, we investigated the effects of COX-2 on VEGF production in gastric cancer cell lines and compared its effects with those in gastric fibroblasts.

Materials and Methods

Materials

N-(2, cyclohexyloxy-4-nitrophenyl) methane sulfonamide (NS-398) was kindly donated by Taisho Pharmaceutical Co. (Tokyo, Japan). Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse anti human COX-2 antibody (IBL, Gunma, Japan) and horseradish peroxidase (HRP) conjugated anti mouse antibody (Promega, Madison, WI, USA) were used for Western blot analysis.

Cell Culture

Human gastric fibroblasts GF02 were prepared from gastric tissues resected at surgery from patients with gastric carcinomas. Briefly, resected tissues were immediately washed three times with isotonic NaCl solution, and the submucosal tissue was separated from the mucosa and cut into 1～2 mm pieces. After washing with Dulbecco's modified essential medium (DMEM : Sigma), the tissue samples were suspended in DMEM supplemented with 10% FBS (Biosciences PTY LTD, Lornevic, Australia) and placed in 100 mm culture dishes. The fibroblasts were allowed to adhere to the dishes and grow in a humidified atmosphere of 5% CO_2 at 37°C. We confirmed that these cells were fibroblasts by immunostaining with a monoclonal antibody against vimentin (Dako). Only the third- or fourth-passage fibroblasts were used throughout present experiments. The human gastric fibroblast cell line, Hs 262.St, which was derived from human gastric ulcer tissue, was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). It was maintained in RPMI-1640 (Nikken, Tokyo, Japan) containing 10% FBS, penicillin-streptomycin (Gibco BRL, Tokyo, Japan) and cultured in a humidified atmosphere of 5% CO_2 in air at 37°C.

Human gastric cancer cell lines, MKN1 (adenosquamous carcinoma), MKN28 (tubular adenocarcinoma), MKN74 (tubular adenocarcinoma), NUGC-3, AZ-521 and JR-1 were kindly supplied by the Japanese Cancer Research Resources Bank. TMK-1 (poorly differentiated adenocarcinoma) was a generous gift from Dr. Hiroshi Yokosaki (First Department of Pathology, Hiroshima University, Hiroshima). These cells were maintained in RPMI-1640 containing 10% heat inactivated FBS, penicillin-streptomycin and allowed to adhere to the dishes and grow in the same conditions as indicated above.

Preparation of culture supernatant

GF02 and Hs 262.St cells were plated onto 24 well culture plates at a concentration of 10^4/well. After 24-hour incubation at 37°C, the monolayers were washed twice with PBS and cultured in medium without FBS after additional an 24 hours and were washed twice with PBS again. The cells were then incubated in each medium containing FBS at various concentrations in the presence or absence of NS-398 (10 μM) or indomethacine (10 μM) for 24 hours. The culture supernatants were collected and stored at −20°C before measurement of VEGF and PGE2.

Hs 262.St cells were stimulated by FBS at concentration gradient of 0, 2.5, 5, 10, 20, 40 (%). GF02 cells were stimulated by FBS at the final concentration of 2.5%. MKN1, MKN28, MKN74, TMK-1, JR-1, NUGC-3, and AZ521 cells were seeded onto 24-well culture plates, and stimulated with 10% FBS. After 24 hours, each supernatant was harvested and stored at −20°C until assay. Cells were seeded onto the 6 cm culture dishes to harvest cell lysates.

Measurement of VEGF and PGE2

Amounts of VEGF protein in the culture conditioned media were measured by sandwich enzyme-linked immunosorbent assay (ELISA) with a human VEGF ELISA kit (IBL) according to the manufacturer's instruction. The VEGF antibody used in the kit was specific for $VEGF_{165}$.

The content of PGE2 in the media was measured directly using a PGE2 EIA kit (Cat. No. 90001, Assay Designs, Inc, Jones, Ann Arbor, MI, USA).

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of COX-2 and VEGF mRNA

Expression of VEGFmRNA transcripts were detected with RT-PCR as described previously[10]. Briefly, cells were lysed with 350 μl of lysis buffer containing guanidinium isothiocyanate (RNeasy Kit, Qiagen, Tokyo, Japan). From the cell lysate, the total RNA was extracted according to the manufacturer's instructions. RT-PCR was performed with 2 μg of total RNA in a total volume of 25 μl of 10 mmol/lTris Hcl buffer, pH 8.3, containing 500 mmol/l deoxynucleotide triphosphate (dNTP) mixture, 4 nmol random primer and 8 U of murine leukemia virus reverse transcriptase (Sawady, Tokyo, Japan) and was then reverse transcribed at 37°C.

For amplification, PCR was performed exactly as described previously[10]. COX-2 mRNA was detected as described previously[9]. β-actin was amplified using the same conditions as in VEGF amplification except that the annealing temperature was set at 61°C. The amplified PCR products were electrophoresed on 1% agarose gel containing ethidium bromide.

Western blot analysis

Expression of COX-2 protein in gastric fibroblasts and cancer cells was detected with Western blot analysis as described previously[11]. Briefly, cells were harvested in 25 nmol/L Tris-HCL (pH 8.1) buffer, containing 0.25 mol/L sucrose, 1.0 mmol/L phenylmethylsulfonyl fluoride, 1.0 μmol/L pepstatin A, and 1.0 mmol/L ethylenediaminetetraacetic acid. The pellet was collected by centrifugation at 10,000 g for 2 minutes and resuspended in the same buffer. 3-〔(3-Cholanmidopropyl) dimethylammonio〕-1-prooanesulfonate (CHAPS : Sigma) was added to 1% (wt/vol), and the mixture was stirred for 2 hours at 4°C. After centrifugation at 50,000 g for 20 minutes, the supernatant was loaded onto an anion-exchange column equilibrated with 20 mmol/L Tris-HCL (pH 8.1) plus 0.4% CHAPS. Protein was determined using a DC protein assay kit as described in manufacturer's instruction (Bio-Rad Laboratories, Hercules, CA, USA).

Samples containing 50 μg of protein were separated on 10% acrylamide gel by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In the next step, the proteins were electrophoretically transferred to a nitrocellulose membrane and probed with anti-COX-2 antibody specific for human COX-2 protein. Bound antibodies were detected with HRP-conjugated antibodies using an enhanced chemiluminescence detection system.

Statistical analysis

Results are shown as means±SE from triplicate determination. Statistical significance was determined with Student t-test and one-way ANOVA ; a P value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Expression of VEGF in human gastric fibroblasts and cancer cells

We first tested whether human gastric fibroblasts and cancer cell lines could produce VEGF in culture media only supplemented with 10% FBS. Primary human gastric fibroblasts GF02 and gastric cancer cell lines MKN1 and MKN74 were cultured in media supplemented with 10% FBS. Before and 24 hours after the culture, the media were collected and VEGF protein was measured by ELISA. Twenty four hours' culture of GF02 and two gastric cancer cell lines promoted an increase of VEGF concentration in the media, though the levels were different (Fig. 1). MKN1 and MKN74 showed significant expression of VEGF.

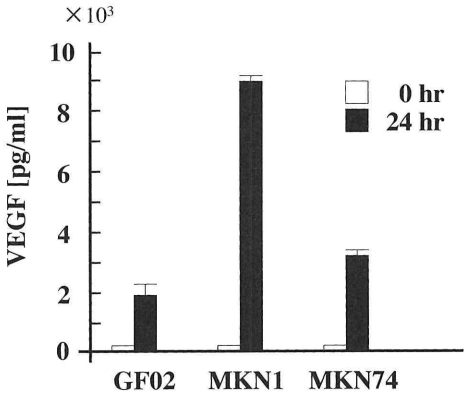

Fig. 1 The amount of VEGF in supernatant of human gastric fibroblasts (GF02) and gastric cancer cells before and 24 hours after cell culture in media supplemented with 10% FBS.

The effects of indomethacin and NS-398 on VEGF production in human gastric fibroblasts

Secondly we tested the effects of FBS on VEGF production by other gastric fibroblasts and also examined the dose dependency. Human gastric fibroblasts, Hs 262.St, were inoculated onto 24-well culture plates at a concentration of 10^5/well. Then the confluent cells were stimulated for 24 hours by FBS at different concentrations : 0, 2.5, 5, 10, 20, 40%. Fig. 2 shows that in Hs262.St the VEGF production was also stimulated by FBS and that the level of VEGF increased dose dependently up to 40%. Serum free media did not stimulate VEGF production by fibroblasts.

Next we examined the effects o f indomethacin and the selective COX-2 inhibitor, NS-398, on VEGF pro-

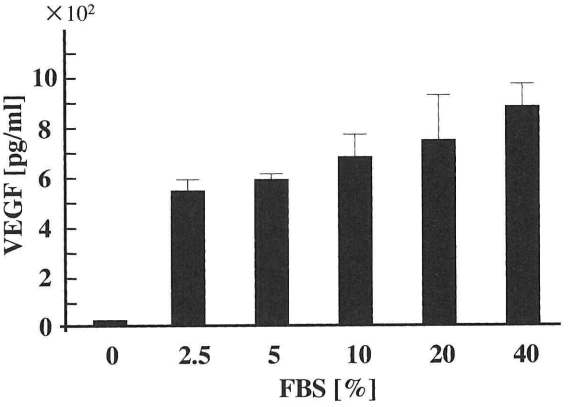

Fig. 2 The amount of VEGF in the supernatant of human gastric fibroblasts (Hs262.St) cultured in various concentrations of FBS.

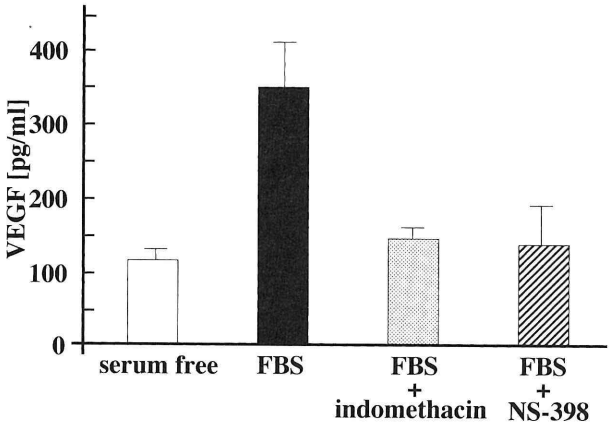

Fig. 3 Effects of indomethacin and NS-398 on VEGF production in human gastric fibroblasts cultured in RPMI 1640 containing 10% FBS

duction, to know whether the production depends on prostaglandin synthesis. VEGF expression of Hs 262. St was suppressed by either indomethacin or NS398 at a concentration of 10 μM (Fig. 3). VEGF production by GF02 was also inhibited by indomethacin and NS-398 (data not shown). The suppressive effects of VEGF production by indomethacin or NS-398 in Hs 262. St was similar. The findings indicated that the VEGF production depends on COX-2 rather than COX-1.

VEGF mRNA expression of human gastric cancer cells

In order to investigate whether splicing variants of VEGF differed among gastric cancer cell lines, we tested the expression of a variety of gastric cell lines derived from different pathological types. Fig. 4 shows the result of RT-PCR analysis of VEGF mRNA expression. VEGF expression of NUGC-3 appeared to be relatively weak, but all of the cell lines expressed four kinds of splicing variants, three of which, that is 400 bp, 520 bp, 650 bp corresponded to $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{201}$, respectively[10]. Expression patterns were similar in the different pathological cell types.

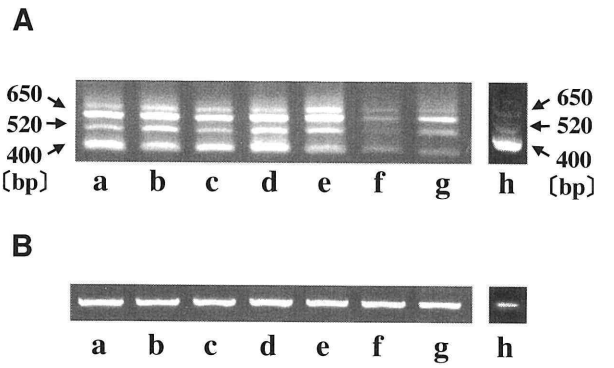

Fig. 4 VEGF (**A**) and β-actin (**B**) mRNA levels of cultured gastric cancer cell lines and human gastric fibroblasts detected in RT-PCR. Each lane indicates a transcript of a : MKN1, b : MKN28, c : MKN74, d : TMK-1, e : JR-1, f : NUGC-3, and g : AZ521 h : Hs262.St.

The effects of Indomethacin and NS-398 on VEGF production in human gastric cancer cells

Cells of the human gastric cancer cell lines MKN1, MKN28, MKN74, TMK1 and JR-1 were detached by 0.025% trypsin EDTA solution and seeded on 24-well culture plates at a concentration of 3×10^5/well. After obtaining confluent culture, cells were starved for 24 hours and cultured with or without 10% FBS in the presence or absence of indomethacin [10 μM] or NS-398 [10 μM]. All of the gastric cancer cell lines produced $VEGF_{165}$ in the culture supernatant, but in none of these was the production suppressed by indomethacin or NS-398 (Fig. 5). These facts suggested that VEGF production was independent of COX expression in these lines (Fig. 5). The expression patterns were quite different from those of gastric fibroblasts.

COX-2 mRNA and protein expression of human gastric cancer cells

To confirm the COX-2 expression of human gastric fibroblasts, we first tested the mRNA expression of these cells by RT-PCR analysis. Fig. 6A shows the expression of COX-2 mRNA by human gastric cancer cell lines : MKN1, MKN28, MKN74, TMK-1, JR-1, NUGC-3 and AZ521. All of these cell lines expressed COX-2 mRNA with β-actin transcripts. Fig. 6B shows the expression of COX-2 protein by human gastric cancer cell lines : MKN1, MKN28, JR-1 and NUGC-3 were apparently expressed the COX-2 protein. In spite of the expression of COX-2 mRNA by RT-PCR analysis, the level of the band of MKN74 and TMK-1 was slightly different from that in the positive control by Western bloting. Because measurement revealed the PGE_2 in the culture supernatant of these cells, we considered that COX-2 protein also exists in MKN74 and TMK-1 cells to some extent, even if we could not detect it in the same level by Western blot analysis (data not shown). This means that indomethacin and NS-398 could not suppress VEGF production, though MKN74 and TMK-1 released PGE2. Taken together, these results indicated that gastric cancer cells produce VEGF independent of COX expression.

The effects of high concentration of indomethacin or NS-398 on VEGF production in human gastric cancer cells

Since the level of the COX-2 expression in most gastric cancer cells appeared higher than that of gastric fibroblasts, we also examined the effects of higher amount of indomethacin or NS-398 on VEGF produc-

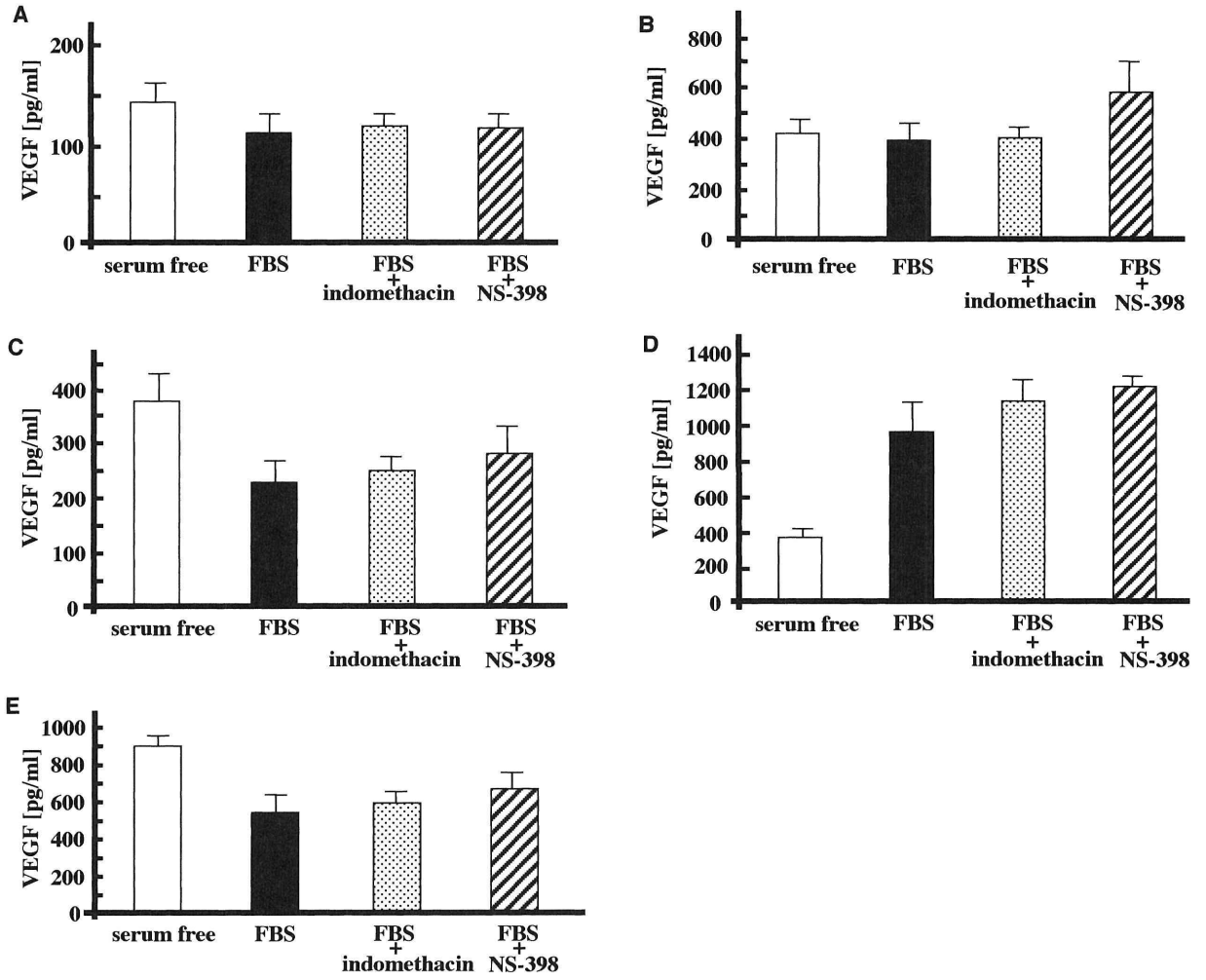

Fig. 5 Effects of indomethacin or NS-398 on VEGF production in human gastric cancer cell lines cultured in RPMI 1640 containing 10% FBS. **A** : MKN1, **B** : JR-1, **C** : MKN74, **D** : MKN28, **E** : TMK-1

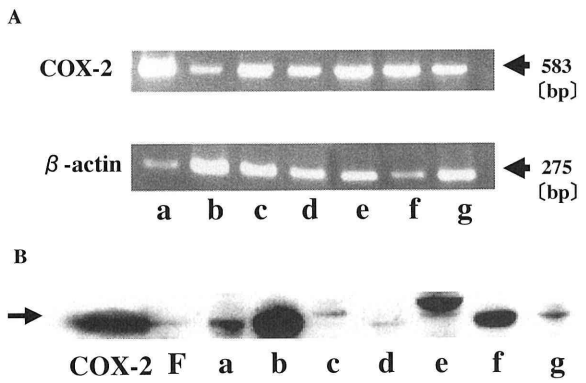

Fig. 6 **A** : Expression of COX-2 mRNA in gastric cancer cell lines. Each line indicates the results of RT-PCR of a : MKN1, b : MKN28, c : MKN74, d : TMK-1, e : JR-1, f : NUGC-3, g : AZ521.
B : Expression of COX-2 protein in gastric cancer cell lines. Each lane indicates the results of Western blotting of F : fibroblast, a : MKN1, b : MKN28, c : MKN74, d : TMK-1, e : JR-1, f : NUGC-3, and g : AZ521. An arrow (→) shows the level of molecular weight for 72 kD.

tion of the cancer cells in serum free condition. VEGF production of gastric cancer cells was not inhibited even in the presence of 100 μM of indomethacin or NS-398 in most of these cells we tested. Furthermore, VEGF production of MKN1 cells was significantly up-regulated by higher concentrations of indomethacin or NS-398, such as 100 μM (Fig. 7A). MKN74 cells also produced significantly higher amounts of VEGF when they were cultured in the presence of 100 μM of NS-398 (Fig. 7B).

In the case of JR-1 cells, VEGF production was slightly inhibited in the presence of NS-398, but it was not significant (Fig. 7C). Administration of PGE_2 [1 μM] in the culture did not alter VEGF production of MKN1 or MKN74 cells, and did not restore VEGF production of JR-1 cells. Similar effects were observed in media containing 10% FBS, but effects were more obvious in serum-free media.

Taken together, these results suggested that the inhibition of COX-2 did not decrease VEGF production in

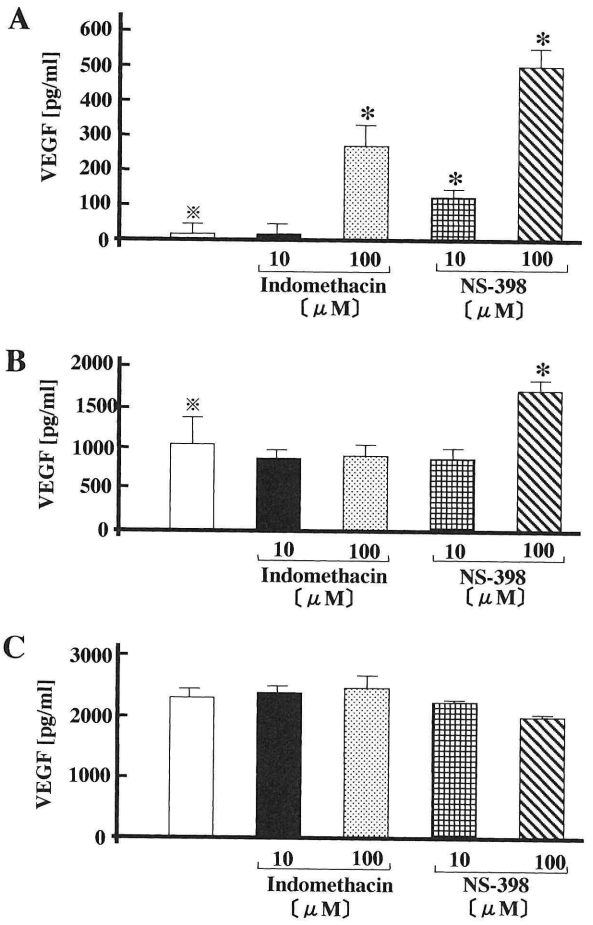

Fig. 7 Effects of indomethacin or NS-398 on VEGF production in human gastric cancer cell lines ($1\text{-}3\times10^5$/well) in serum-free media (**A**: MKN1, **B**: MKN74, **C**: JR-1). Each column shows mean±SE (bar) of VEGF concentration in triplicate culture. ※ vs * shows significant difference according to the statistical analysis described in Materials and Methods ($p<0.05$).

most gastric cancer cells we tested.

This implies that gastric cancer cells have an independent pathway of COX-2 expression to produce VEGF.

Discussion

In this study, we examined whether human gastric cancer cells could produce VEGF, and also investigated the involvement of COX protein in VEGF production. We also compared the VEGF production with that of gastric fibroblasts, to test differences with cellular mechanism of the production. We showed that: (1) cultured gastric cancer cells produced $VEGF_{165}$, (2) RT-PCR analysis showed most of gastric cancer cell lines, we tested, expressed at least 2 other splicing variants of VEGF, (3) the expression of VEGF in these cells was independent of COX-2 expression, (4) high concentration of indomethacin or NS-398 enhanced VEGF production in a part of gastric cancer cell lines, (5) VEGF expression in gastric fibroblasts was quite dependent of that of gastric fibroblasts even when cultured under similar conditions.

COX-2 expression in tumor cells is thought to enhance the production of angiogenic factors such as VEGF, because Tsujii et al. clearly demonstrated that human colon cancer cells increased the production of angiogenic factors if they overexpressed COX-2. However it is not yet clear whether the phenomenon holds true in all colon cancer cells expressing COX-2, and which kind of colon cancers can produce VEGF if they overexpress COX-2. Many tumor cells were also reported to express COX-2, and in some of these COX-2 expression correlated with VEGF production or microvessel density on histological examination[10,11]. To the best of our knowledge, however, no paper has challenged the molecular mechanism and relationship between COX-2 expression and angiogenic factors in these tumors besides colon cancers.

On the other hand, although epidemiological studies and animal studies have suggested that COX-2 may play an important role in colorectal tumorigenesis, recent papers showed that colon cancer dose not always express COX-2 in human tissues, while mesenchymal cells such as macrophages significantly expressed COX-2 in colon adenoma tissue[13,14]. Williams et al also demonstrated that COX-2 expressing Lewis lung tumor cells did not grow in COX-2 knockout mice. These data indicated that COX-2 expressed in stromal cells may be more important than that of cancer cells with regard to their growth in vivo[15].

Although many factors are involved in angiogenesis, VEGF is one of the most potent and specific growth factors for endothelial cells. Data in this study, however, showed that gastric cancer cell lines could express COX-2 and release prostaglandins, but COX-2 inhibitor did not inhibit the VEGF production of these cells. This means that gastric cancer cells do not stimulate VEGF production, at least in an autocrine manner through prostaglandin synthesis, though prostaglandins released from cancer cells might stimulate stromal cells to produce growth factors. It is interesting that some of the gastric cancer cell lines could produce VEGF even in a starved condition. The cellular mechanism of VEGF induction was not clarified in detail, but hypoxia is known to induce VEGF gene expression via hypoxia-inducible factor 1 (HIF-1)[16]. Other factors which up-regulate VEGF production are hypoglycemia and cytokines, such as interleukin (IL)-1β, IL-6, FGF-4, PDGF, tumor necrotizing factor (TNF)-α, TGF-β and insulin like growth factor (IGF)-I [2]. Inactivation of the von Hippel-Lindau tumor suppressor gene was also reported to up-regulate VEGF production of human carcinoma cells, and mutation of p53 is known to enhance VEGF production of tumor cells[17,18].

Eicosanoids also influenced VEGF production. PGE_1 and E_2 are reported to stimulate VEGF production in osteoblasts, fibroblasts and macrophages[19]. A recent paper showed 15-deoxy-(12, 14)-prostaglandin J(2) (PGJ2) augmented VEGF production of monocytes and endothelial cells[20]. The reason why cyclooxygenase inhibitors did not down-regulate VEGF production in gastric cancer cells is not yet clear. It is of interest why the high amount of indomethacin and NS-398 enhanced VEGF production in these cells. Most gastric cancers we tested expressed the same size of splicing variants in RT-PCR analysis. $VEGF_{165}$ is the major isoform of secreted VEGF, though a significant fraction remains bound to the cell surface and extracellular matrix. $VEGF_{189}$, and $VEGF_{206}$ bind to heparin with greater affinity than $VEGF_{165}$[21]. Gastric fibroblasts also expressed splicing variants similar to cancer cells.

We do not know whether non-steroidal anti-inflammatory drugs (NSAIDs) can prevent the growth of tumors other than colorectal tumors. Even if colonic tumors which express COX-2 might be a possible target for cancer prevention or therapy with NSAIDs, our data indicated that a selective COX-2 inhibitor did not suppress the VEGF production of any pathological type of cancer cells, thus selective COX-2 inhibitors might not be enough for clinical use against gastric cancers. A recent paper demonstrated that administration of selective COX-2 inhibitor and MAPK inhibitors could induce the reduction of tumor growth, though selective COX-2 inhibitor is not as effective by itself[22]. Further investigations are necessary to determine molecular mechanism of VEGF production and tumor growth in gastric cancers.

Conclusion

Primary cultured gastric fibroblasts could produce VEGF in the media only supplemented bovine calf serum, and the production was inhibited by indomethacin or NS-398 〔10 μM〕. However, indomethacin or NS-398 did not inhibit the production of VEGF in gastric cancer cell lines, though those cell lines could express COX-2 mRNA. These results suggested VEGF production of gastric cancer cells might be involved in different mechanism from COX-2 expression.

Acknowledgements

We thank Ms. Hideko Yamagishi for her excellent technical help.

Referernces

1) Folkman J : Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. **1** : 27-31, 1995
2) Ferrara N, Davis-Smyth T : The biology of vascular endothelial growth factor. Endocr Rev. **18** : 4-25. Review. No abstract available, 1997
3) Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN : Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell. **93** : 705-716, 1998
4) Cianchi F, Cortesini C, Bechi P, Fantappie O, Messerini L, Vannacci A, Sardi I, Baroni G, Boddi V, Mazzanti R, Masini E : Up-regulation of cyclooxygenase 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. Gastroenterology. **121** : 1339-1347, 2001
5) Uefuji K, Ichikura T, Mochizuki H : Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. Clin Cancer Res. **6** : 135-138, 2000
6) Gately S : The contributions of cyclooxygenase-2 to tumor angiogenesis. Cancer Metastasis Rev. **19** : 19-27, 2000
7) Folkman J : Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. **1** : 27-31, 1995
8) Shweiki D, Itrin, A, Soffer D, Keshet E : Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature **359** : 843-845, 1992
9) Tatsuguchi A, Sakamoto C, Tsukui T, Fukuda Y, Kobayashi M : Cyclooxygenase-2 is associated with angiogenesis and apoptosis in gastric cancer. Gastroenterology. **120**(5 Suppl 1) : P-4
10) Ramakrishnan R, Zell JA, Malave A, Rathinavelu A : Expression of vascular endothelial growth factor mRNA in GI-101A and HL-60 cell lines. Biochem Biophys Res Commun. **270** : 709-713, 2000
11) Kirschenbaum A, Liu X, Yao S, Levine AC : The role of cyclooxygenase-2 in prostate cancer. Urology. **58**(2 Suppl 1) : 127-131. Review, 2001
12) Uotila P, Valve E, Martikainen P, Nevalainen M, Nurmi M, Harkonen P. Increased expression of cyclooxygenase-2 and nitric oxide synthase-2 in human prostate cancer. Urol Res. **29** : 25-28, 2001
13) Bamba H, Ota S, Kato A, Adachi A, Itoyama S, Matsuzaki F : High expression of cyclooxygenase-2 in macrophages of human colonic adenoma. Int J Cancer. **83** : 470-475, 1999
14) Fujita M, Fukui H, Kusaka T, Ueda Y, Fujimori T : Immunohistochemical expression of cyclooxygenase (COX)-2 in colorectal adenomas. J Gastroenterol. **35** : 488-490, 2000
15) Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN : Host cyclooxygenase-2 modulates carcinoma growth. J Clin Invest. **105** : 1589-1594, 2000
16) Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL : Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol. **16** : 4604-4613, 1996
17) Siemeister G, Weindel K, Mohrs K, Barleon B, Martiny-Baron G, Marme D. Reversion of deregulated expression of vascular endothelial growth

factor in human renal carcinoma cells by von Hippel-Lindau tumor suppressor protein. Cancer Res. **15**: 56: 2299-2301, 1996
18) Arii S, Mori A, Uchida S, Fujimoto K, Shimada Y, Imamura M: Implication of vascular endothelial growth factor in the development and metastasis of human cancers. Hum Cell. **12**: 25-30, 1999
19) Harada S, Nagy JA, Sullivan KA, Thomas KA, Endo N, Rodan GA, Rodan SB: Induction of vascular endothelial growth factor expression by prostaglandin E2 and E1 in osteoblasts. J Clin Invest. **93**: 2490-2496, 1994
20) Inoue M, Itoh H, Tanaka T, Chun TH, Doi K, Fukunaga Y, Sawada N, Yamshita J, Masatsugu K, Saito T, Sakaguchi S, Sone M, Yamahara Ki, Yurugi T, Nakao K. Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-gamma. Arterioscler Thromb Vasc Biol. **21**: 560-566, 2001
21) Houck KA, Leung DW, Rowland AM, Winer J, Ferrara N: Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. J Biol Chem. **267**: 26031-26037, 1992
22) 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. **6**: 1024-1028, 2000

胃線維芽細胞と胃癌細胞での血管内皮増殖因子 (VEGF) 産生に対する COX-2 の役割の相違性

三浦崇幣[1)] 津久井拓[2)] 篠木啓[2)]
竹山裕樹[1)] 赤松知憲[2)] 三宅一昌[2)]
和田謙[2)] 溝上裕士[1)] 小林正文[2)]
松岡健[1)] 坂本長逸[2)]

[1)]東京医科大学内科学第五講座
[2)]日本医科大学内科学第三講座

【要旨】 近年血管内皮特異的な増殖因子，vascular endothelial growth factor (VEGF) の存在が報告され，さまざまな病態において新生血管の形成に関与することが明らかになった．VEGF は強力な angiogenic factor として知られており，腫瘍の増殖と密接に関連すると考えられている．大腸癌細胞を用いた最近の研究では，VEGF の産生に prostaglandin 合成の律速酵素である cyclooxygenase (COX) が密接に関与することが報告されており，その subtype である COX-2 は大腸癌の VEGF 産生を増加させることが報告されている．一方，胃癌細胞における COX-2 の発現と VEGF 産生の関連を検討した報告は少ない．我々は培養胃癌細胞を FBS の存在下に培養し，① 7 種類の胃癌樹立細胞株における VEGF mRNA の発現を検討し，全ての細胞に $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$ の 3 種類の splicing variant を確認した．② 胃癌樹立細胞株の培養上清中に存在する $VEGF_{165}$ を測定し，VEGF の産生を確認した．③ 胃癌樹立細胞株には，少なくとも 5 種に COX-2 蛋白の発現を認めたが，その VEGF 産生は indomethacin, NS-398 を用いても抑制できなかった．④ 胃癌細胞株の一部は高濃度の indomethacin, NS-398 によって無血清下でむしろ VEGF の産生が亢進した．⑤ 同様にヒト胃線維芽細胞を FBS 存在下に培養すると VEGF の産生が認められる．しかし，この反応は indomethacin および NS-398 によって抑制された．これらの結果から胃癌細胞の VEGF 産生は胃線維芽細胞とは異なり，COX-2 を介さず異なる機序が関与している可能性が示唆された．

〈**Key words**〉 Cyclooxygenase-2，胃線維芽細胞，胃癌細胞，VEGF