Gastric Epithelial Cells Secrete A PDGF-like Peptide, a Potent Mitogen for Human Gastric Fibroblasts (44212)

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Abstract. **To investigate whether gastric epithelial cells secrete growth factors involved in stromal cell growth, we examined the effects of conditioned media obtained from gastric cancer cells on murine BALB/c 3T3 cells and primary cultured human gastric fibroblasts. Conditioned media from MKN-1 gastric cancer cells were applied to a heparin-affinity column. The fraction eluted from the column at 0.4 M NaCl stimulated DNA synthesis and phosphorylation of PDGF α-receptors on tyrosine in BALB/c 3T3 cells. The fraction-induced stimulation of DNA synthesis in gastric fibroblasts was more marked than in BALB/c 3T3 cells. However, the fraction failed to stimulate DNA synthesis in CHO-ER cells overexpressing EGF receptors and phosphorylation of PDGF β-receptors on tyrosine in BALB/c 3T3 cells. Immunoblot analysis of the media confirmed that PDGF-AA-like peptides are released from gastric cancer cells, immortalized gastric epithelial cells, and primary cultured gastric epithelial cells. Anti-PDGF neutralizing antibodies produced only a partial inhibition of 0.4 M NaCl fraction-induced enhanced DNA synthesis. Thus, in addition to PDGF-AA peptide, other bioactive substance(s) are probably released from MKN-1 gastric cancer cells. Our results suggest that gastric epithelial cells secrete PDGF-AA-like peptides responsible for stromal cell growth through paracrine mechanisms.** [P.S.E.B.M. 1998, Vol 217]

Proliferation of gastric epithelial cells in response to various growth factors is important for maintaining mucosal integrity and acceleration of peptic ulcer healing. Several growth factors, such as the epidermal growth factor (EGF), transforming growth factor-α (TGFα), heparin binding EGF-like growth factor (HB-EGF), and hepatocyte growth factor (HGF) are known to induce mitogenic response in primary cultured gastric epithelial cells (1, 2, 3). These growth factors are localized in the gastric epithelial and stromal cells and exert their effects on the proliferation, migration and differentiation of epithelial cells as autocrine or paracrine factors. Proliferation and migration of epithelial cells are important processes, and development of mucosal stroma is essential for the healing of peptic ulceration. Accumulating evidence suggests that stromal cells, such as fibroblasts, play an important role in the regulation of gastric epithelial cell growth and migration (3, 4). However, very little is known about the mechanisms regulating the growth of mucosal stroma and granulation tissue in peptic ulceration.

The stroma is composed of fibroblasts, vascular tissue, (e.g., endothelial and smooth muscle cells), and a variety of extracellular matrix proteins. In general, stromal cells are thought to be regulated by a variety of growth factors, such as the platelet-derived growth-factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor-β (TGFβ), produced by stromal cells themselves or by macrophages. However, keratinocytes, the epithelial cells of the epidermis, are the major source of PDGF, which is a principal mitogen for stromal cells and thought to play an important role in skin wound healing (5). Recent studies have also shown that PDGF mRNA is expressed in other tissues such as renal epithelial cells (6, 7). Thus, it seems that the interaction between epithelial and stromal cells is important.

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Based on the findings in other tissues, it is also possible that gastric epithelial cells may also regulate the growth of stromal tissue through paracrine mechanisms.

To investigate whether gastric epithelial cells secrete growth factors involved in stromal cell growth, we examined the effects of conditioned media obtained from gastric cancer cells on murine BALB/c 3T3 cells and primary cultured human gastric fibroblasts derived from macroscopically normal gastric mucosa of patients with gastric cancer. Our results showed that gastric cancer cells, as well as cultured gastric epithelial cells, may secrete PDGF-AA-like peptide. The peptide enhanced the growth of primary cultured gastric fibroblasts more markedly than BALB/c 3T3 cells.

Materials and Methods

Materials. Recombinant human PDGF-AA, -BB, and anti-human PDGF neutralizing antibodies were purchased from Becton Dickinson Labware (Bedford, MA). Anti-human PDGF single A chain antibodies and anti-human PDGF single B chain antibodies were obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-phosphotyrosine antibodies (PY-20) were purchased from ICN (Costa Mesa, CA). Rabbit antiserum against PDGF α- and β-receptors were kindly provided by Dr. T. Matsui (The Third Department of Internal Medicine, Kobe University School of Medicine). Recombinant TGFα was purchased from Oncogene Science, Inc. (Uniondale, NY). Recombinant basic FGF (bFGF) was kindly supplied by Takeda Pharmaceutical Co. (Osaka, Japan).

Cell Lines. Cell lines established from human gastric cancer tissues (MKN-1, MKN-28, MKN-45, TMK-1, KATO III, KWS, JR-1) and BALB/c 3T3 mouse fibroblast cell line were kindly supplied by the Japanese Cancer Research Resources Bank. Gastric cancer cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS), streptomycin, and penicillin G, while BALB/c 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with the same serum and antibiotics. Chinese hamster ovary cells transfected with the full-length human EGF receptor cDNA and overexpressing the receptors (CHO-ER cells) were cultured as described previously (8). A gastric mucosa cell line established from transgenic mice harboring temperature-sensitive simian Virus 40 large T-antigen gene (GSM06) was kindly provided by Daiichi Pharmaceutical Co. (Tokyo, Japan). GSM06 cells were maintained as described previously by Sugiyama *et al.* (9).

Primary Culture. Human gastric fibroblasts 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 DMEM, the tissue samples were suspended in DMEM supplemented with 10% FCS 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 in air 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 the present experiments. Gastric mucous epithelial cells from guinea pig were also prepared and cultured as described previously by our laboratory (10).

Preparation of Conditioned Media. Confluent MKN-1 gastric cancer cells in 100-mm culture dishes were rinsed twice with phosphate-buffered saline (PBS) and cultured for another 24 hr in DME/F12 medium (5 ml/dish) without FCS. Thereafter, the conditioned medium was collected, centrifuged at 1500 × *g* for 15 min, and the supernatant was filtered and stored at −20°C prior to use. In experiments examining the effects of unpurified conditioned media, the conditioned medium was concentrated into 10% of the initial volume using Centricon-3 microconcentrator (Amicon, Beverly, MA) and then dialyzed against 20 mM *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)/NaOH (pH 7.4) containing 10 μg/ml aprotinin, 1 mM benzamidine and 10 μM (*p*-amidinophenyl)-methanesulfonyl fluoride at 4°C for 12 hr.

Thymidine Incorporation Assay. [^{3}H]thymidine incorporation assay was performed as described previously with a slight modification (11). Briefly, confluent BALB/C 3T3 cells or primary cultured gastric fibroblasts on 96-well plates were starved with serum-free medium for 24 hr. The cells were then incubated with aliquots of either concentrated conditioned media or column eluates for 18 hr. In the next step, 1 μCi of [^{3}H]thymidine (Amersham) was added to each well, and the cells were incubated for another 4 hr. [^{3}H]thymidine incorporation into the cells was measured by a liquid scintillation counter.

Heparin-affinity Purification. Conditioned media (150–200 ml) from MKN-1 cells were applied to a heparin-affinity column (Econo-Pac heparin cartridge, Bio-Rad) equilibrated with 20 mM HEPES/NaOH (pH 7.4) on an HPLC system. Heparin binding molecules bound to the column were eluted with a linear gradient of 0.15–2.0 M NaCl in 20 mM HEPES/NaOH. Fractions (2 ml) were collected, and aliquots (10 μl) were assayed for the level of DNA synthesis in either BALB/c 3T3 cells or gastric fibroblasts.

Characterization of Growth Stimulatory Activity. The biophysical properties of heparin-binding molecules were characterized by boiling the column eluates for 10 min or treating them with 5 mM dithiothreitol (DTT) for 2 hr at 37°C. The fraction was then dialyzed against 20 mM HEPES/NaOH. The effects of these samples were examined by [^{3}H]thymidine incorporation into cells.

Immunoblot Analysis. The active fraction (100 μl) eluted from the heparin-affinity column was lyophilized, solubilized with the Laemmli's sample buffer, boiled, and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. To confirm the presence of PDGF A chains in media condi-

tioned from various gastric cancer cells (MKN-28, -45, TMK-1, KATO III, KWS, and JR-1), GSM06, and primary cultured guinea pig gastric epithelial cells without column chromatography, 2% sodium deoxycholate (12.5 μl) were added into each conditioned medium (1.5 ml). The mixture was incubated on ice for 15 min, followed by the addition of 0.5 ml of 24% trichloroacetic acid (TCA), and the sample was centrifuged at 3300 × *g* for 30 min. The precipitate was washed with diethyl ether, solubilized with the Laemmli's sample buffer, boiled, and subjected to SDS-PAGE. Proteins separated by SDS-PAGE were transferred onto nitrocellulose sheets as described previously (12), and immunoblot analysis was performed using the ECL detection system (Amersham).

PDGF Receptor Phosphorylation. Confluent BALB/c 3T3 cells were serum-starved for 24 hr and stimulated for 5 min at 37°C with PDGF-AA, -BB, or bioactive fractions that had been dialyzed against DMEM. The cells were immediately frozen in liquid nitrogen and stored at −80°C until lysis. The cells were lysed in a solution containing 10 mM tris(hydroxymethyl)aminomethane/HCl (pH 7.4), 150 mM NaCl, 1% NP40, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM EDTA, 1 mM vanadate, 10 μg/ml aprotinin, and 1 mM benzamidine. Clear lysates containing 1.5 mg of protein were incubated with 3 μl of rabbit anti-serum against PDGF α-receptor or β-receptor coupled with protein G sepharose (Pharmacia) for 90 min at 4°C. Immunoprecipitates were washed with a solution containing 50 mM HEPES/NaOH (pH 7.4), 150 mM NaCl, and 0.1% Triton X-100 and then boiled for 5 min in Laemmli's sample buffer. The samples were then subjected to SDS-PAGE and transferred onto nitrocellulose sheets. Immunoblot analysis was performed using PY20.

Statistical Analysis. All values were expressed as mean ± SEM. Differences were examined for statistical significance using the Student's *t*-test. A *p* value of less than 5% denoted the presence of statistical significance.

Results

Bioactivity of Conditioned Media of Gastric Cancer Cells. In the first step, we compared the effects of media conditioned from MKN-1 gastric cancer cells with those of various growth factors on [^{3}H]thymidine uptake in BALB/c 3T3 cells. Conditioned media of MKN-1 gastric cancer cells increased [^{3}H]thymidine uptake. Although PDGF-AA at 50 ng/ml caused a weak stimulation, TGFα, bFGF, and PDGF-BB resulted in more than a 6-fold increase in [^{3}H]thymidine uptake in BALB/c 3T3 cells (Fig. 1). The bioactivity in the media increasing [^{3}H]thymidine uptake in BALB/c 3T3 cells was eluted from heparin affinity resin at approximately 0.4 M NaCl (Fig. 2), suggesting that the bioactive substances contain heparin-binding molecules.

Bioactivity of 0.4 M NaCl Fraction. We then examined the bioactivity of 0.4 M NaCl fraction using CHO-ER cells overexpressing human EGF-receptors, primary

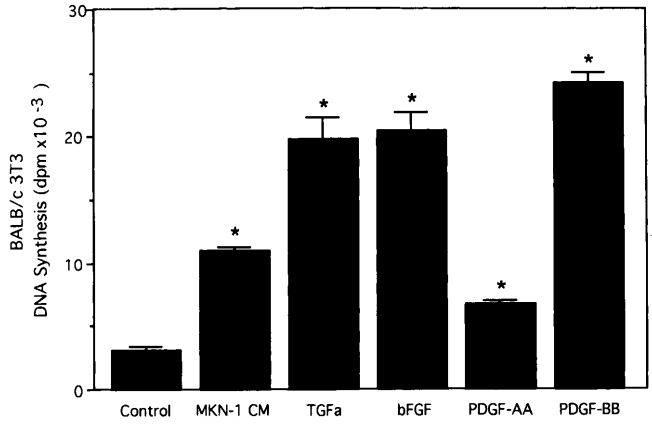

Figure 1. Effects of media conditioned from MKN-1 gastric cancer cells on DNA synthesis. The conditioned medium was prepared from confluent MKN-1 gastric cancer cells as described in the Methods. The effects of the conditioned medium on [^{3}H]thymidine uptake were compared with those of 10 ng/ml TGFα, 10 ng/ml bFGF, 50 ng/ml PDGF-AA or 50 ng/ml PDGF-BB in BALB/c 3T3 cells. Values shown are mean ± SEM of triplicate determinations from a representative of three separate experiments. *P < 0.01 compared with control.

cultured gastric epithelial cells, immortalized GSM06 gastric epithelial cells, and MKN-1 cells. The 0.4 M NaCl fraction did not exert any bioactivity itself to stimulate [^{3}H]thymidine uptake in these cells, whereas TGFα at 10 ng/ml or 10% FCS strongly increased [^{3}H]thymidine uptake into these cells (data not shown). These results suggest that the 0.4 M NaCl fraction may contain growth factors for stromal cells but not for epithelial cells.

The bioactive substances in the 0.4 M NaCl fraction were not EGF-like or FGF-like growth factors that are usually eluted at more than 0.6 M NaCl from heparin affinity resin (13, 14, 15). Furthermore, the bioactivity necessary to stimulate [^{3}H]thymidine uptake in BALB/c 3T3 cells disappeared when 5 mM of DTT was added to the fraction. On

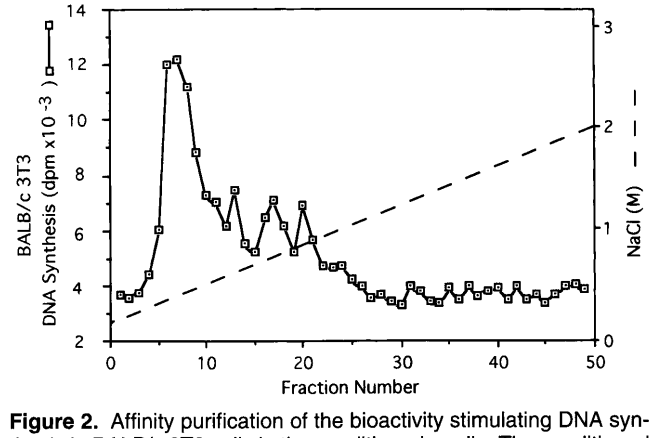

Figure 2. Affinity purification of the bioactivity stimulating DNA synthesis in BALB/c 3T3 cells in the conditioned media. The conditioned medium obtained from MKN-1 gastric cancer cells was applied to a heparin-affinity column. Heparin binding molecules bound on the column were eluted with a linear gradient of 0.15–2.0 M NaCl, and the bioactivity to stimulate [^{3}H]thymidine uptake in each fraction was examined in BALB/c 3T3 cells. Values are the mean of triplicate determinations from a representative example of two separate experiments.

the other hand, the bioactivity was stable even after boiling for 10 min (data not shown). Based on these results, it is possible that the bioactive substance in the 0.4 M NaCl fraction is either a PDGF-homodimer or a heterodimer. When anti-PDGF neutralizing antibodies were added at a concentration that usually completely inhibits the effect of 50 ng/ml PDGF-AA or when PDGF-BB, the antibodies produced a partial reduction of the stimulatory effect of the 0.4 M NaCl fraction on DNA synthesis; this suggested that PDGF-like peptides may be, at least in part, involved in the bioactivity of the 0.4 M NaCl fraction (Fig. 3). When recombinant PDGF-AA or -BB was applied to the heparin-affinity column, its bioactivity was also eluted at the 0.4 M NaCl fraction (data not shown). However, the addition of 50 ng/ml PDGF-AA or -BB to the 0.4 M NaCl fraction produced a partial enhancement of the stimulatory effect on [^{3}H]thymidine uptake in BALB/c 3T3 cells (data not shown). In our experiments, 50 ng/ml of PDGF-AA or -BB was the maximal effective dose for BALB/c 3T3 cells. These results suggest that the 0.4 M NaCl fraction may also contain other bioactive substance(s) for fibroblasts.

PDGF Receptor Phosphorylation by 0.4 M NaCl Fraction. In order to determine whether PDGF receptors are phosphorylated on tyrosine in response to the 0.4 M NaCl fraction, BALB/c 3T3 cells were treated with either PDGF-AA, -BB or the 0.4 M NaCl fraction alone. PDGF α-receptor immunoprecipitates that were blotted by anti-phosphotyrosine antibodies showed that the NaCl fraction stimulated tyrosine phosphorylation of the receptors (Fig. 4A). On the other hand, PDGF β-receptor immunoprecipitates blotted by anti-phosphotyrosine antibodies showed that the NaCl fraction was not effective in phosphorylation of PDGF β-receptors on tyrosine (Fig. 4B).

Immunoblotting with anti-PDGF A or anti-PDGF B chain antibodies in 100 μl of lyophilized 0.4 M NaCl fraction showed that the fraction contained only PDGF A chain-like peptides (Fig. 5A), and no PDGF B chains (Fig. 5B). The latter were not detected even when 1 ml of 0.4 M NaCl fraction was lyophilized and immunoblotted with anti-PDGF B chain antibodies (data not shown).

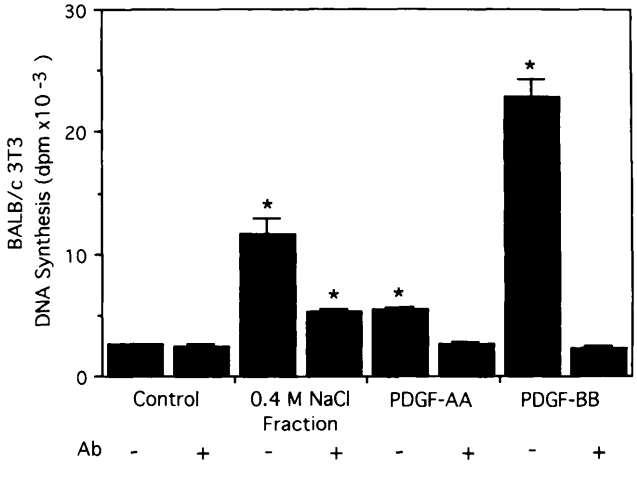

Figure 3. Effects of anti-PDGF neutralizing antibodies on 0.4 M NaCl fraction-induced DNA synthesis in BALB/c 3T3 cells. Anti-PDGF neutralizing antibodies were used at a concentration that completely inhibited the biological activity of 50 ng/ml PDGF-AA or 50 ng/ml PDGF-BB. Values are mean ± SEM of triplicate determinations from a representative example of two separate experiments. *P < 0.05 compared with control.

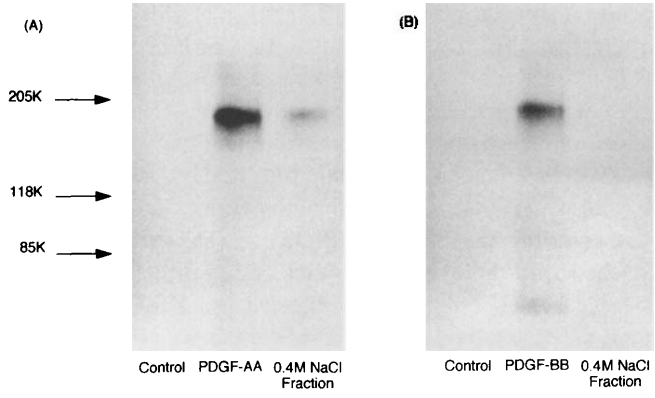

Figure 4. Effects of 0.4 M NaCl fraction on PDGF α- or β-receptor phosphorylation on tyrosine in BALB/c 3T3 cells. Serum-starved BALB/c 3T3 cells were incubated with or without 50 ng/ml PDGF-AA, 50 ng/ml PDGF-BB, or 0.4 M NaCl fraction for 5 min at 37°C. Cell lysates, prepared as described in the Methods, were immunoprecipitated by either anti-serum against PDGF α-receptor (A) or anti-serum against PDGF β-receptor (B). Immunoprecipitates were subjected to SDS-PAGE, transferred onto nitrocellulose sheets, and immunoblotted by anti-phosphotyrosine antibodies. Control represents unstimulated samples.

Effects of 0.4 M NaCl Fraction and PDGF-AA on Human Gastric Fibroblasts. We also examined the effects of the 0.4 M NaCl fraction on DNA synthesis by using primary cultured gastric fibroblasts. The NaCl fraction caused a 3.3-fold increase in [^{3}H]thymidine uptake in hu-

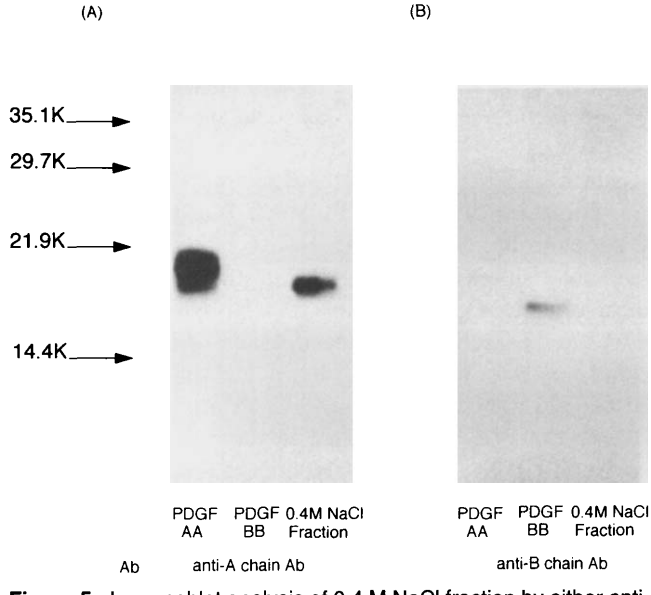

Figure 5. Immunoblot analysis of 0.4 M NaCl fraction by either anti-PDGF A chain or anti-PDGF B chain antibodies. 100 μl of 0.4 M NaCl fraction and 10 ng of recombinant PDGF-AA or PDGF-BB in 100 μl HEPES/NaOH were lyophilized, solubilized with Laemmli's sample buffer, and subjected to SDS-PAGE. The samples were transferred onto nitrocellulose sheets and immunoblotted by anti-PDGF A chain (A) or anti-PDGF-B chain (B) antibodies.

man gastric fibroblasts. PDGF-AA also produced more than a 4-fold increase in DNA synthesis (Fig. 6). Comparison with the effects of 50 ng/ml PDGF AA in BALB/c 3T3 cells (Fig. 1) showed that the responsiveness to PDGF-AA was more marked in human gastric fibroblasts. Furthermore, the bioactivity of the 0.4 M NaCl fraction in human gastric fibroblasts was also partially inhibited by anti-PDGF neutralizing antibodies (data not shown).

Immunoblotting of PDGF A Chains in Media from Other Gastric Cells. Using immunoblotting, we examined the presence of PDGF A chains in media of other gastric cancer cells as well as immortalized or primary cultured gastric epithelial cells. In six different gastric cancer cell lines examined, not including MKN-1, PDGF A chains were detected in two cell lines: MKN-45 and KATO III. In addition, immortalized GSM06 gastric epithelial cells and primary cultured guinea pig gastric epithelial cells also secreted PDGF A chain-like peptides (Fig. 7). Although the bands immunoblotted by anti-PDGF A chain antibodies were somewhat smaller in size than PDGF A chain-like peptides found in human gastric cancer cells, the peptides were not visualized under nonreducing conditions (data not shown). Since the antibodies used in our experiments did not interact with the A-chain dimer, but with the monomer, the results suggest that these peptides are probably PDGF A chain-like peptides similar to those found in human gastric cancer cells.

Discussion

The major findings of the present study were that PDGF-AA-like peptides were secreted from gastric cancer cells as well as cultured gastric epithelial cells and that the secreted PDGF-AA-like peptides and probably other

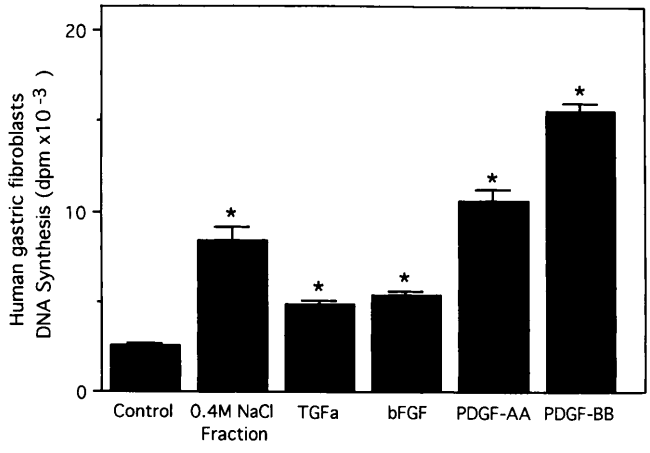

Figure 6. Effects of 0.4 M NaCl fraction and various growth factors on DNA synthesis in primary cultured human gastric fibroblasts. Human gastric fibroblasts were obtained and cultured as described in the Methods. The effects of 0.4 M NaCl fraction on [^{3}H]thymidine uptake were examined in human gastric fibroblasts, and the results were compared with those of 10 ng/ml TGFα, 10 ng/ml bFGF, 50 ng/ml PDGF-AA or 50 ng/ml PDGF-BB. Values are mean ± SEM of triplicate determinations from a representative of two separate experiments. $P < 0.01$ compared with control.

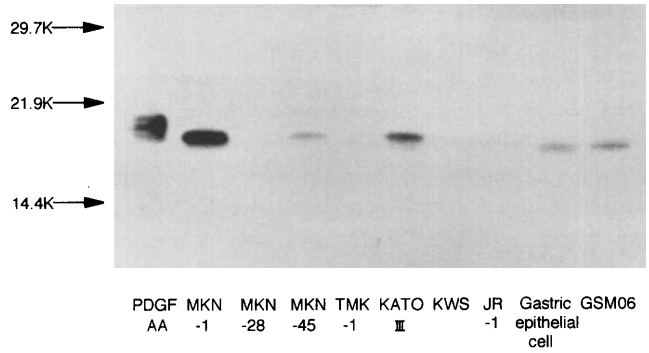

Figure 7. Immunoblot analysis of PDGF A chain in conditioned media from various gastric cancer or epithelial cells. Conditioned media were prepared from several gastric cancer cells (MKN-1, MKN-28, MKN-45, TMK-1, KATO III, KWS, JR-1), primary cultured gastric epithelial cells and GSM06 mouse gastric epithelial cells. Proteins in the medium (1.5 ml) were precipitated with 12.5 μl of 2% sodium deoxycholate followed by 0.5 ml of 24% TCA, washed with diethyl ether, and solubilized with the Laemmli's sample buffer. The samples were subjected to SDS-PAGE, transferred onto nitrocellulose sheets, and immunoblotted by anti-PDGF A chain antibodies.

yet unknown growth factor(s) have stimulatory effects on DNA synthesis in stromal fibroblasts through a paracrine mechanism.

Our results also showed that media conditioned from MKN-1 cells had stimulatory effects on DNA synthesis in BALB/c 3T3 cells, and the bioactivity was eluted at the 0.4 M NaCl fraction when the conditioned medium was applied onto the heparin-affinity column. Several lines of evidence obtained in the present study suggest that this bioactivity may be due, at least in part, to PDGF-AA itself. First, the bioactivity in the 0.4 M NaCl fraction was heat-stable but disappeared under reducing conditions, suggesting that the bioactive substances contain disulfide bonds that are critical for the observed bioactivity. PDGF-AA or -BB, a recombinant dimeric peptide, was also eluted at the same fraction in our experimental systems. Second, anti-PDGF antibodies that produced a total neutralization of the stimulatory effects of recombinant PDGF-AA and -BB on BALB/c 3T3 cells also neutralized approximately 65%–75% of the bioactivity eluted at 0.4 M NaCl. Third, the NaCl fraction stimulated phosphorylation of PDGF α-receptors but not PDGF β-receptors in BALB/c 3T3 cells. It should be noted that not only homodimer peptides (e.g., PDGF-AA and -BB) but also a heterodimer peptide (PDGF-AB) binds to PDGF α-receptors. However, PDGF-BB and PDGF-AB bind to PDGF β-receptors only. Therefore, phosphorylation of the PDGF α-receptor only suggests that the 0.4 M NaCl fraction contains PDGF-AA-like peptides. Moreover, PDGF A and not PDGF B chain-like peptides were detected in the 0.4 M NaCl fraction by immunoblotting using specific anti-PDGF A and B chain antibodies. These results confirmed that this fraction contained PDGF A chain-like peptides. Thus, our results suggest that a bioactive PDGF-AA peptide was probably released into the media from MKN-1 gastric cancer cells.

In the present study, MKN-1 gastric cancer cells were the main cell types used to obtain conditioned media. This

limitation is due to unavailability of 100% pure immortalized human gastric epithelial cells. However, our results suggest that immortalized GSM06 mouse gastric epithelial cells and primary cultured gastric epithelial cells of guinea pigs may also secrete PDGF-AA-like peptides into the culture media. Immunoblot analysis showed that the molecular weight of PDGF A chain-like peptide in GSM06 and guinea pig gastric epithelial cells was approximately 1 KDa, smaller than that found in cancer cells. The smaller splice-variant forms are similar to those secreted by normal human umbilical vein endothelial cells (16). Furthermore, the biochemical characteristics of the smaller peptide suggest that it may also be a PDGF A chain-like peptide similar to that found in human gastric cancer cells.

Previous studies have shown gene and peptide expression of PDGF A and B chains in a variety of cell lines (17, 18, 19). In particular, high levels of PDGF A chain mRNA are present in many gastric cancer cells (20). However, it remains to be established whether bioactive PDGF-AA is actually secreted from gastric cancer cells. By using immunoblot analysis, we confirmed that a PDGF-AA-like peptide was secreted and that it stimulated tyrosine phosphorylation of PDGF α-receptors. Furthermore, by using human gastric fibroblasts, we found that the peptide released from gastric cancer cells acted on stromal cells derived from the same organ. More importantly, the effect of recombinant PDGF-AA on primary cultured gastric fibroblasts was more marked than in BALB/c 3T3 fibroblasts. In addition to these results, previous reports have also suggested that skin epithelial cells are the major source of PDGF (5). Since there was no evidence that PDGF receptor expression was present in cultured keratinocytes, these studies suggest that PDGF may play a paracrine role in stromal cell growth in the skin. PDGF A and B chain expression have been detected in the lung, mammary, and gastric cancer cells (17, 21). Furthermore, the expression of PDGF A and B chain mRNA as well as PDGF A or B chain immunoreactivity have been also shown in normal mammary and renal epithelial cells and biopsy specimens from gastric ulcers (6, 7, 22, 23, 24). Combined together, the results of our study and those of other investigators suggest that the PDGF-AA-like peptide secreted from gastric epithelial cells may play a crucial role in gastric mucosal stromal cell growth through paracrine mechanisms.

In addition to PDGF-AA, MKN-1 gastric cancer cells may secrete other yet unknown growth factor(s) for stromal cells. This conclusion is based on our finding that neutralizing antibodies, known to produce a total inhibition of the stimulatory effect of recombinant PDGF on DNA synthesis, did not cause a complete suppression of the stimulatory effects of the 0.4 M NaCl fraction on DNA synthesis in BALB/c 3T3 cells. Furthermore, the 0.4 M NaCl fraction enhanced the effect of PDGF-AA or -BB on DNA synthesis. A likely candidate producing such an effect is the heparin binding growth factor, but such a factor could not belong to the family of EGF-like growth factors, such as HB-EGF, amphiregulin, and betacellulin, since the 0.4 M NaCl fraction did not stimulate DNA synthesis in CHO-ER cells overexpressing human EGF receptors. On the other hand, the activity is not likely to be due to bFGF since this factor is normally eluted at more than 1.0 M NaCl from a heparin-affinity column (25). Further studies are necessary to characterize the exact type of other possible growth factors present in the 0.4 M NaCl fraction.

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