Expression of c-sis/Platelet-derived Growth Factor B, Insulin-like Growth Factor I, and Transforming Growth Factor α Messenger RNAs and Their Respective Receptor Messenger RNAs in Primary Human Gastric Carcinomas: In Vivo Studies with in Situ Hybridization and Immunocytochemistry.¹

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ABSTRACT

In situ hybridization and immunocytochemistry have been applied to investigate the expression of c-sis/platelet-derived growth factor (PDGF)-B, insulin-like growth factor (IGF)-I, and transforming growth factor α messenger RNAs and their respective receptor messenger RNAs in three primary human gastric carcinomas and in their adjacent nonmalignant mucosas. Expression of c-sis/PDGF-B mRNA and PDGF-receptor β mRNA was seen in the tumor cells of the three gastric cancer specimens but not in their adjacent nonmalignant mucosa. The mRNA expression was accompanied by the expression of their respective protein products. IGF-I, IGF-I receptor, and epidermal growth factor receptor mRNA were seen in both the tumor cells of the gastric cancer specimens and in nonmalignant mucosa. Transforming growth factor α mRNA was expressed in gastric tumor cells but not in nonmalignant mucosa. The expression of TGF-α mRNA which was seen only in the tumor were expressed in both malignant and nonmalignant tissues. Similarly, IGF-I and the type I IGF receptor were expressed in both malignant and nonmalignant tissues. The major difference between normal and malignant tissues appears to be the expression of c-sis/PDGF-B and PDGF-R β mRNAs, and that of TGF-α mRNA which was seen only in the tumor cells of the cancer specimens. These findings suggest the presence of potent in vivo autocrine loops of “competence” and “progression” growth factors in the gastric tumor cells contributing to their unregulated growth.

INTRODUCTION

Gastric cancer is associated with high mortality with less than 15% of patients surviving for 5 years. In the United States it represents 0.5 to 1.0% of all causes of death. In contrast, gastric cancer is common in Japan and Korea, and in certain parts of China, Africa, and Central and South America. The age-adjusted mortality rate from gastric cancer in the United States is 2.7% of all cancer death, while in Korea and Japan they are 36 and 25%, respectively (1-3). Dietary factors have been implicated in the high frequency of gastric cancer. However, a link between these factors and the molecular events leading to the development of gastric cancer is unknown.

Studies in cultured cell lines derived from human gastric cancer and in gastric cancer tissue specimens have shown the expression of several genes encoding for growth factors and growth factor receptors. These studies were based primarily on the demonstration of gene expression by Northern blot analysis, using for this purpose RNA extracted from cultured cells or primary tissues. Results from these studies have shown the expression of TGF-α (4, 5) and its receptor, the EGF-R, in both human gastric cancer and in normal nonmalignant mucosa (5-8). Higher amounts of EGF-R mRNA appeared to be expressed in the tumor specimen than in normal gastric tissue (5). Similar results were reported on the expression of TGF-β with higher expression seen in gastric carcinoma than in normal tissue (9). The expression of PDGF-A mRNA was reported in both gastric cancer specimens and in normal mucosa (10). PDGF receptor β mRNA was also detected in both cancer and normal specimens. In contrast PDGF-B mRNA could not be detected in these specimens (10). Expression of IGF-I receptor has been reported in two gastric tumor cell lines (7).

In the present studies we used in situ hybridization for the demonstration of the expression of specific mRNAs in primary gastric tumors and adjacent nonmalignant gastric mucosa. This approach enables not only the demonstration of specific mRNA expression but it also allows to establish the types of cells in the primary tumors expressing the mRNA. In situ immunocytochemistry was used to demonstrate the expression of protein products.

We report here the expression of the c-sis/PDGF-B and PDGF-R β mRNAs in the tumor cells of human gastric carcinomas but not in adjacent nonmalignant gastric mucosa. TGF-α mRNA was expressed only in tumor cells but its receptor, the EGF-R mRNA was expressed in both tumor cells and the normal mucosa. Similarly, IGF-I and the type I IGF receptor were expressed in both malignant and nonmalignant tissues. The major difference between normal and malignant tissues appears to be the expression of c-sis/PDGF-B and PDGF-R β mRNAs, and that of TGF-α mRNA which was seen only in the tumor cells of the cancer specimens. These findings suggest the presence of potent in vivo autocrine loops of “competence” and “progression” growth factors in the gastric tumor cells contributing to their unregulated growth.

MATERIALS AND METHODS

Tissue Specimen. The specimens used in this study were obtained from patients with primary gastric carcinoma, hospitalized and operated on in the Chunchon Sacred Heart Hospital, Chunchon, Korea. The specimens were collected intraoperatively, frozen immediately in liquid nitrogen, and stored at −80°C. Three primary gastric adenocarcinoma specimens with adjacent nonmalignant mucosa from each specimen serving as control were investigated in these studies.

In Situ Hybridization. Sections (2 mm thick) from tumors or adjacent tissues were immersed in ice-cold 4% paraformaldehyde for 2–8 h and then were allowed to stand in 30% sucrose/phosphate-buffered saline overnight at 4°C to decrease freezing artifacts. The fixed tissues were then embedded in O.C.T. (Miles Laboratories, Inc., Naperville, IL) for cryostat serial sectioning (8 μm) (150 to 200 sections/tissue). The tissue sections were subjected to in situ hybridization, utilizing 35S-labeled complementary RNA probes (11). The specificity of the in situ hybridization was controlled by hybridization of serial sections with control, noncomplementary (sense) RNA probes. Duplicate sections from each tissue were hybridized with either complementary antisense or noncomplementary sense probes and were developed at weekly intervals for over a period of 2 weeks. Data presented here are from a 2-week exposure.

The cDNA probes for these studies include c-sis/PDGF-B (12),

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⁴ The abbreviations used are: TGF, transforming growth factor; EGF-R, epidermal growth factor receptor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; CEA, carcinoembryonic antibody.
RESULTS

Expression of c-sis/PDGF-B and PDGF-R β mRNAs. Fig. 1 shows the strong expression of c-sis/PDGF-B mRNA in the tumor cells of a primary human gastric adenocarcinoma tissue that was counterstained with CEA (Fig. 1A). In contrast, epithelial cells in adjacent nonmalignant tissue did not express c-sis mRNA (Fig. 1B). The expression of c-sis mRNA in the gastric tumor cells was accompanied by the expression of the PDGF-R β mRNA (Fig. 1C). There was no significant expression of PDGF-R β mRNA in the nonmalignant adjacent tissue (Fig. 1D). Control in situ hybridization of the malignant tissues with noncomplementary sense riboprobes for c-sis mRNA or PDGF-R β mRNA produced negative results (data not shown).

Expression of PDGF-like and PDGF-R-like Proteins. Fig. 2 shows the expression of PDGF-like proteins (Fig. 2A), and PDGF-R β-like protein (Fig. 2B) in the tumor cells of primary gastric adenocarcinoma. This suggests that the c-sis and PDGF-R β mRNAs expressed by the tumor cells (Fig. 1) are functional mRNAs translating into their respective protein products. The specificity of the immunostaining reaction was controlled by preincubation of the PDGF antiserum with excess PDGF-B homodimer (50 ng) (Fig. 2C), and the PDGF-R β antiserum with excess synthetic receptor peptide (100 ng) (Fig. 2D). Under these control conditions there was no detectable immunostaining for PDGF-like or PDGF-R β-like proteins.

Expression of IGF-I and Type I IGF Receptor mRNAs. As shown in Fig. 3, IGF-I mRNA was expressed in both the tumor cells of gastric carcinoma tissue (Fig. 3A) and in adjacent mucosa (Fig. 3C). The specificity of the IGF-I mRNA expression in the tumor cells (Fig. 3A) was controlled by parallel hybridization of tumor sections using noncomplementary sense riboprobe for IGF-I mRNA (Fig. 3B). There was no significant expression with the sense riboprobe. Similarly, control hybridization of nonmalignant gastric mucosa with the sense riboprobe produced negative results (Fig. 3D).

The expression of IGF-I mRNA in tumor and nonmalignant gastric epithelial cells was accompanied by the coexpression of the type I IGF receptor I mRNA (Fig. 4). A strong expression of IGF-I receptor mRNA can be seen in the tumor cells of gastric carcinoma tissue that was counterstained with CEA (Fig. 4D). Nonmalignant epithelial cells of normal gastric mucosa (Fig. 4C) also expressed type I IGF receptor mRNA. Control hybridization with the sense riboprobe produced negative results both in the tumor specimen (Fig. 4B) and in the nonmalignant gastric tissue specimen (Fig. 4D). The studies presented in Figs. 3 and 4 show that IGF-I and IGF-I receptor mRNAs are expressed in both the tumor cells of gastric carcinomas and in nonmalignant gastric mucosa.

DISCUSSION

The present studies investigated the in vivo expression of growth factor and their respective receptor mRNAs in primary human gastric carcinoma. An unexpected finding in these studies is the demonstration of the expression of c-sis/PDGF-B mRNA and PDGF receptor β mRNAs and their respective protein products in the malignant epithelial cells of the tumor specimen. Normally, epithelial cells do not express PDGF receptors and they do not produce PDGF-like proteins (for review see Ref. 18). As shown in these studies, nonmalignant epithelial cells in normal gastric mucosa did not express c-sis/PDGF-B mRNA and PDGF-R β mRNA. Previous reports have demonstrated the expression of c-sis/PDGF-B mRNA and the production of PDGF-like mitogen in cultured malignant epithelial cell lines derived from human breast (19, 20), prostatic (21) and lung (22) carcinomas. However, there was no information concerning the expression of PDGF receptors by these cultured malignant epithelial cell lines. In contrast, recent in vivo studies in primary human lung carcinomas revealed the coexpression of c-sis/PDGF-B mRNA and PDGF-R β mRNA in the tumor cells of the lung carcinomas (23). Thus, these in vivo studies with in situ hybridization and immunocytochemi-
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Fig. 1. Localization of c-sis/PDGFB mRNA (A) and PDGF-R β mRNA (C) in the tumor cells of a primary human gastric adenocarcinoma by in situ hybridization. Notice the absence of expression of c-sis/PDGFB mRNA (B) and PDGF-R β mRNA (D) in adjacent nonmalignant mucosa. In Fig. 1A the tumor cells of gastric adenocarcinoma were counterstained with CEA. Original magnification, ×630.

Fig. 2. Immunocytochemistry for PDGF-like proteins (A) and PDGF-R β-like proteins (B) in tissue sections of a primary human gastric adenocarcinoma. Arrows indicate areas of immunostaining. The specificity was controlled by immunostaining in the presence of excess c-sis/PDGFB homodimer (50 ng) (C) or excess synthetic receptor polypeptide (100 ng) (D). Original magnification, ×630.
Fig. 3. Localization of IGF-I mRNA expression in both the tumor cells of a primary human gastric adenocarcinoma (A) and in adjacent nonmalignant gastric mucosa (C). The specificity of the expression was controlled by parallel hybridization of the malignant (B) and nonmalignant (D) tissues with noncomplementary sense riboprobe for IGF-I, which produced negative results. Original magnification, × 630.

Fig. 4. Localization of a strong expression of IGF-I receptor mRNA in the tumor cells of gastric adenocarcinoma counterstained with CEA (A) (arrows). The specificity of the expression was controlled by parallel hybridization with control sense riboprobe. Notice the absence of significant hybridization in the control malignant tissue counterstained with CEA (B) (arrow). Adjacent nonmalignant gastric mucosa also expressed IGF-I receptor mRNA (C). Control hybridization of this tissue with sense riboprobe did not show significant expression (D). Original magnification, × 630.
Fig. 5. Expression of TGF-α mRNA in the tumor cells of a primary human gastric adenocarcinoma counterstained with carcinoembryonic antigen (A). Adjacent nonmalignant gastric mucosa did not express significant levels of TGF-α mRNA (B). Macrophages in both the malignant (C) and nonmalignant (D) tissues expressed TGF-α mRNA. Macrophages in the tissue were counterstained with EMB II antibody (arrows). Original magnification, × 630.

Fig. 6. A strong expression for EGF receptor can be seen in both the tumor cells of gastric adenocarcinoma tissue (A) and in adjacent nonmalignant mucosa (B). The tumor cells in (A) were counterstained with carcinoembryonic antigen (arrow). The mRNA expression was accompanied by the expression of EGF-R-like proteins in both the malignant tissue (C) and in the gastric mucosa (D). Original magnification, ×630.
try have revealed the expression of both c-sis/PDGF-B and PDGF-R β mRNAs and their respective protein products in the malignant epithelial cells of primary human gastric and lung carcinomas.

Unlike PDGF and its receptor, IGF-I and IGF-I receptor mRNAs were localized in both malignant epithelial cells of the gastric cancer specimens and in nonmalignant epithelial cells of gastric mucosa. EGF receptor mRNA and its respective protein product was also localized in both malignant and nonmalignant gastric specimens. As suggested by earlier studies (5), EGF receptor mRNA appears to be more abundant in the tumor cells (Fig. 6A) than in nonmalignant mucosa (Fig. 6B). In contrast, TGF-α mRNA was expressed in the tumor cells of the gastric cancer specimens but not in adjacent nonmalignant mucosa. Macrophages in both malignant and nonmalignant tissue expressed TGF-α mRNA. The expression of the specific growth factor and growth factor receptor mRNAs shown above appears to be uniform and can be seen across the tumor sections. In contrast, the protein products were not expressed uniformly by all cells in these sections. This may reflect a reduced sensitivity of the immunostaining process, compared to in situ hybridization, or to the fact that only part of the population of tumor cells express the protein products at a given time.

The important finding in the present studies is that tumor cells, unlike nonmalignant cells, coexpress c-sis/PDGF-B and its receptor, along with IGF-I and its receptor, and TGF-α and its receptor. Previous in vivo studies have shown a strong synergistic action between PDGF and IGF-I or PDGF and TGF-α stimulating epithelial and connective tissue regeneration in skin wounds of experimental animals (24). PDGF alone, IGF-I alone, or TGF-α alone were significantly less effective in stimulating epithelial and connective tissue regeneration in vivo than the combinations of PDGF with IGF-I or PDGF with TGF-α (24). A coordinate action between PDGF and IGF-I (25) and EGF and IGF-I (26) has been shown to influence in vitro the growth of cultured 3T3 cells. If this synergistic action holds true for epithelial cancer cells in gastric tissue, then the presence of powerful growth factor combinations in these malignant cells may contribute to their unregulated growth. Additional support for this possibility is provided by recent in vivo studies that have shown the coexpression of c-sis/PDGF-B and PDGF receptor β mRNAs and their respective protein products in skin epithelial cells of normal animals subjected to acute cutaneous injury (27). Control, uninjured epithelial cells did not express c-sis and PDGF-R β mRNAs. Expression of PDGF and its receptor in the epithelial cells occurred within 1 day of injury and was suppressed by 9 days after injury, at which time there was complete reepithelialization and healing of the injured tissue. It appears that this reversible expression of PDGF and its receptor in skin epithelial cells following acute injury is part of a physiological function that serves for the normal healing and reepithelialization of the injured tissue. We speculate that an abuse of this physiological process may cause the irreversible, inappropriate expression of PDGF and its receptor in malignant epithelial cells contributing to their uncontrolled proliferation. In gastric cancer, such an abuse may result from "chronic injury" caused by "dietary" factors that appear to be responsible for the increased incidence of gastric cancer in Japan, Korea, and certain areas of China, and Central and South America.

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| Table 1 In vivo expression of mRNA in gastric tumor cells and in adjacent gastric mucosa |
|--------------------------------------|-----------------|-----------------|
| mRNA                                | Gastric tumor cells | Control gastric mucosa |
| PDGF-B                              | +                | −               |
| PDGF-R β                            | +                | −               |
| IGF-I                               | +                | +               |
| IGF-I receptor                      | +                | +               |
| TGF-α                               | +                | −               |
| EGF-R                               | +                | +               |


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