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.

Cha K. Chung2 and Harry N. Antoniades3

The Center for Blood Research, and the Departments of Cancer Biology and Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115

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 (TGF-α) mRNAs and their respective receptor mRNAs 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 mRNAs were seen in both the tumor cells of the gastric cancer specimens and in normal mucosa. Transforming growth factor α mRNA was expressed in gastric tumor cells but not in nonmalignant mucosa. The coexpression of a potent “competence” growth factor, PDGF, and “progression” growth factors, IGF-I and transforming growth factor α, in the tumor cells of gastric carcinomas may contribute to their growth and maintenance.

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 in 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 the tumor cells and the normal mucosa. Similarly, IGF-I and 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 Specimens. 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 32P-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), c-sis/PDGF-A (13), IGF-I (14), IGF-I receptor (15), EGF-R (16), and TGF-α (17). The riboprobe cDNA's were synthesized by run-off transcription of cDNA templates in the presence of [32P]UTP in vitro. The riboprobe cDNA's were confirmed to contain the proper size of RNA by gel electrophoresis. The cDNA templates were cloned in the plasmid pBluescript II KS (Stratagene, La Jolla, CA) and were sequenced using a Sequenase kit (United States Biochemical, Cleveland, OH). The riboprobe cDNA's were denatured and then annealed to total RNA extracted from cultured cells or primary tissues using a riboprobe generation kit (Stratagene, La Jolla, CA) as described by the supplier.

Received 11/14/91; accepted 4/3/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grants CA30101 and HL29583 from NIH (H. N. A.) and the Council for Tobacco Research, USA, Inc. (H. N. A.). Supported by a grant from the Korea Science and Engineering Foundation.

2 Supported by a grant from the Korea Science and Engineering Foundation (KOSEF). Dr. Chung is a visiting scholar in the Department of Nutrition, Harvard School of Public Health. Present address: Hallym University, Department of Food and Nutrition, 1 Okchon-Dong, Chunchon 200, Korea.

3 To whom requests for reprints should be addressed, at The Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115.

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.
Expression of IGF-I and Type I IGF Receptor mRNAs. In gastric tumor cells of a primary human gastric adenocarcinoma tissue, the expression of c-sis/PDGF-B mRNA was accompanied by the expression of EGF receptor-like protein in both the tumor and nonmalignant epithelial cells (Fig. 5A). There was no significant expression of c-sis mRNA and PDGF-R β mRNA. Previous reports have demonstrated the expression of c-sis mRNA and PDGF-R β mRNA in the malignant epithelial cells of the tumor specimen (Fig. 5B) and in the nonmalignant gastric tissue specimen (Fig. 5D). 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.

Expression of TGF-α and EGF Receptor mRNAs. Data shown in Fig. 5 demonstrate the expression of TGF-α mRNA in the tumor cells of gastric carcinoma tissue counterstained with carcinoembryonic antibody (Fig. 5A). There was no significant expression of TGF-α mRNA in adjacent control gastric mucosa (Fig. 5B). TGF-α mRNA expression was also seen in macrophages of both malignant (Fig. 5C) and nonmalignant gastric tissues (Fig. 5D). Macrophages were stained (brown) with the EMB-11 antibody as indicated by the arrows in Fig. 5, C and D.

Fig. 6 shows the strong expression of EGF receptor mRNA in the tumor cells of gastric carcinoma tissue (Fig. 6A). In this study, in situ hybridization for EGF receptor mRNA was combined with immunostaining of the tumor cells with carcinoembryonic antibody (Fig. 6A). A strong expression of EGF receptor mRNA was also shown in nonmalignant gastric mucosa (Fig. 6B). The expression of EGF receptor mRNA was accompanied by the expression of EGF receptor-like protein in both the tumor specimen (Fig. 6C) and the control, nonmalignant tissue (Fig. 6D).

A summary of the data is presented in Table 1. The major difference between gastric tumor tissue and normal gastric mucosa is the expression of the c-sis mRNA, PDGF receptor β mRNA, and TGF-α mRNA in the tumor cells but not in nonmalignant gastric mucosa. In contrast, both tumor and nonmalignant cells expressed IGF-I and IGF-I receptor mRNAs and EGF receptor mRNAs. The examples presented in Figs. 1–6 are representative of the findings seen in the tissue sections derived from the three individual human gastric carcinomas specimens, and adjacent 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. It appears that the gastric malignant epithelial cells inappropriately express both c-sis 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 immunocytochemis-
Fig. 1. Localization of c-sis/PDGF-B 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/PDGF-B 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/PDGF-B 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-1 or PDGF and TGF-α in 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.

**ACKNOWLEDGMENTS**

We thank Drs. Young I. Park and Min C. Lee, Department of Pathology, Hallym University, for providing the gastric tissue specimens used in this study for the tissue pathology. The authors wish to acknowledge the generous donation of the human type I receptor complementary DNA clone by Dr. William J. Rutter, and the complementary DNA clone for the human IGF-I by the Institute of Molecular Biology, Boston, MA. Our thanks to Cale List for the preparation of this manuscript.

**REFERENCES**

19. Rosengurt, E., Sinnet-Smith, J., and Taylor-Papadimitrou, J. Production of Table 1 In vivo expression of mRNA in gastric tumor cells and in adjacent gastric mucosa

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Gastric tumor cells</th>
<th>Control gastric mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-B</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PDGF-R β</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IGF-1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IGF-1 receptor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TGF-α</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>EGF-R</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

19. Rosengurt, E., Sinnet-Smith, J., and Taylor-Papadimitrou, J. Production of


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.

Cha K. Chung and Harry N. Antoniades


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/52/12/3453

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.