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Gastrin Receptor Gene Expression in Several Human Carcinomas

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Gastrin has been shown to enhance the growth of various human tumors. The present study was designed to examine the gastrin receptor gene expression in various human carcinoma cell lines and in surgically resected carcinoma tissues. By Northern blot analysis, gastrin receptor mRNA was detected in 3 out of 7 small cell lung carcinoma cell lines. Gastrin receptor mRNA was also expressed in one out of 8 colon carcinoma cell lines and 2 out of 10 colon carcinoma tissues. Moreover, one of two small cell carcinoma cell lines of the stomach clearly expressed gastrin receptor mRNA. However, none of the gastric adenocarcinoma cell lines or surgically resected gastric adenocarcinomas tested had any detectable expression of gastrin receptor gene. These findings may suggest a role of gastrin receptor in the growth and differentiation of certain human carcinomas.

Key words: Gastrin receptor — Small cell lung cancer — Small cell gastric cancer — Gastric cancer — Colon cancer

Gastrin plays an important role in the gastric phase of acid secretion. In addition, several lines of evidence suggest that gastrin exerts trophic effects not only on normal gastrointestinal mucosa,[1,2] but also on various human tumors.[3–6] Indeed, patients with hypergastrinemia due to Zollinger-Ellison syndrome as well as type-A chronic atrophic gastritis occasionally develop carcinoid tumor of the stomach.[7,8] Furthermore, binding studies have revealed the presence of gastrin receptors in gastric as well as colon cancer cell lines.[9,10] In addition, gastrin has been shown to enhance the growth of various human carcinomas such as small cell lung cancers (SCLCs),[3] colorectal cancers,[4,11] and gastric cancers,[5,12] both *in vivo* and *in vitro*. Supporting these observations, we have recently cloned gastrin receptor cDNA from the gastric enterochromaffin-like (ECL) carcinoid tumor of *Mastomys natalensis*.[13] Moreover, we observed that gastrin significantly enhanced the growth of CHO cells transfected with human gastrin receptor cDNA.[14]

Sethi *et al.*[15] have demonstrated expression of the gastrin receptor gene in some human SCLC cell lines. However, no data have yet been obtained to indicate whether or not gastric or colon cancers express gastrin receptor gene. In the present study, therefore, in addition to SCLC, we examined the presence of gastrin receptor gene expression not only in human gastrointestinal cancer cell lines, but also in surgically resected cancer tissues.

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MATERIALS AND METHODS

Cell lines Seven human SCLC cell lines, 14 gastric carcinoma cell lines (5 poorly differentiated adenocarcinomas, 6 well differentiated adenocarcinomas, 2 small cell gastric carcinomas, 1 of undetermined origin), and 8 colon carcinoma cell lines were used for this study. SCLC cell lines, Lu134A, Lu134B, Lu135, Lu139, PC6 and PC14, and gastric carcinoma cell lines, HGC27, HSK-TC and GCIY were supplied by Riken Cell Bank (Tsukuba). An SCLC cell line, H60, was provided by Prof. S. Maeda (Department of Pathology, Kobe University). Gastric carcinoma cell lines MKN1, MKN7, MKN28, MKN45, MKN74, TMK1, KATOIII and OKAJIMA were purchased from Immunological Biochemical Laboratory (Gunma). A gastric cancer cell line AGS and colon cancer cell lines COLO201, COLO205, COLO320DM, COLO320HSR, DLD1, HT29, LoVo and SW403 were purchased from Dainippon Seiyaku Co. Ltd. (Osaka). ECC10 and ECC12 were established from small cell gastric carcinomas of Japanese patients.[16]

Surgical specimens of gastric and colon cancer tissues Ten colorectal and 8 gastric carcinoma tissues, histologically verified, were obtained at surgery. In addition, normal gastric as well as colonic mucosae, from areas in close proximity to the cancer tissues, were also obtained. Immediately after removal, the tissues were stored at −80°C until RNA extraction.

RNA extraction and Northern blot analysis The total RNA was obtained from both the confluent cultured cells and surgically resected tissues by extraction with guani-

dine thiocyanate (GTC), followed by cesium chloride centrifugation. The RNA was separated by electrophoresis on 0.66 *M* formaldehyde-1% agarose gel with 0.4 *M* 3-(N-morpholino)propane sulfonic acid, 0.1 *M* sodium acetate and 0.02 *M* EDTA. The nucleic acid was transferred to nitrocellulose membranes (Schleicher & Schuell, Germany), and nucleic acid was permanently fixed to the membrane by using ultraviolet cross-linking. The probe used for Northern blot analysis was a fragment of human gastrin receptor cDNA (approx. 1.7 kb), which we have recently cloned.[14] Radiolabeled DNA probes were synthesized using a random prime labeling kit (Boeringer-Mannheim, Germany). Hybridization was carried out at 42°C and the filters were washed twice for 20 min at 55°C in 0.1×SSC/0.1% sodium dodecyl sulfate (SDS), as described previously.[17]

Sequencing of gastrin receptor cDNA Total RNA (5 μg) extracted from ECC10 was used to synthesize a single-strand cDNA by the use of a Super Script pre-amplification system (Bethesda Research Laboratories Inc., Gaithersburg, MD). The single-strand cDNA was then amplified directly by the polymerase chain reaction (PCR) method with several 17-mer primers synthesized according to the sequence of the gastrin receptor cDNA of human brain.[14] The amplified cDNA fragments were sequenced by a Sequenase Version 2.0 (United States Biochemical, Cleveland, OH).

DNA Synthesis assay DNA synthesis was measured in terms of the incorporation of [^{3}H]thymidine (Amersham, Buckinghamshire, England) into DNA. Cells were inoculated on 96-well culture plates. When the cells reached confluence in wells, the medium was changed to RPMI1640 containing various concentrations of human gastrin I (Protein Research Foundation, Osaka). After incubation for 24 h at 37°C, [^{3}H]thymidine was added (final concentration: 1 μCi/ml). The cultured cells were incubated for a further 4 h and then washed with 6% trichloracetic acid followed by washing with ethanol. Cells were then lysed with 1 *N* NaOH/0.1% SDS solution and their radioactivity was counted in a liquid scintillation counter.

RESULTS

A major band, which could hybridize with the labeled 1.7-kb fragment of human gastrin receptor cDNA, was detected in 3 out of 7 SCLC cell lines, H60,[18] Lu134A[19] and Lu139[20] (Fig. 1). The molecular size of these bands was approximately 2.3 kb, similar to that of human gastrin receptor mRNA of the brain.[14]

On the other hand, only one out of 8 colon carcinoma cell lines LoVo cells,[21] and 2 of 10 colorectal carcinoma tissues expressed the gastrin receptor transcript; gastrin receptor gene expression was not detected in any of the respective normal colon tissues (Figs. 2 and 3). In contrast, no gastrin receptor mRNA was detected in 12 gastric adenocarcinoma cell lines tested (data not shown). Furthermore, gastrin receptor gene expression was observed in none of the 8 gastric adenocarcinoma tissues tested whereas the surrounding normal gastric mucosa clearly expressed gastrin receptor mRNA when the carcinoma was located in the fundus (sample num-

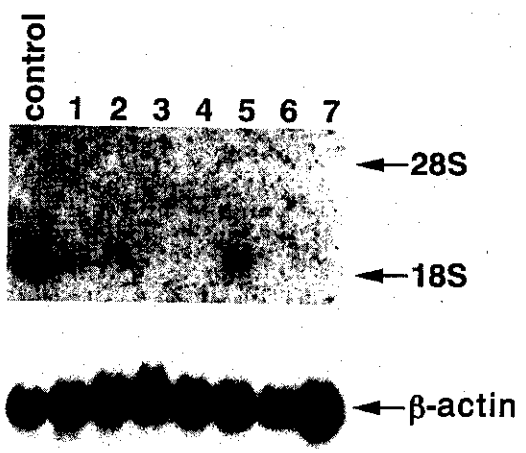

Fig. 1. Northern blot analysis of gastrin receptor mRNA expression in seven small cell lung cancer cell lines. A total RNA gel blot containing 20 μg per lane was hybridized with ^{32}P-labeled probes of human gastrin receptor and β-actin. Control, human fundic mucosa; lane 1, H60; lane 2, Lu134A; lane 3, Lu134B; lane 4, Lu135; lane 5, Lu139; lane 6, PC6; and lane 7, PC14.

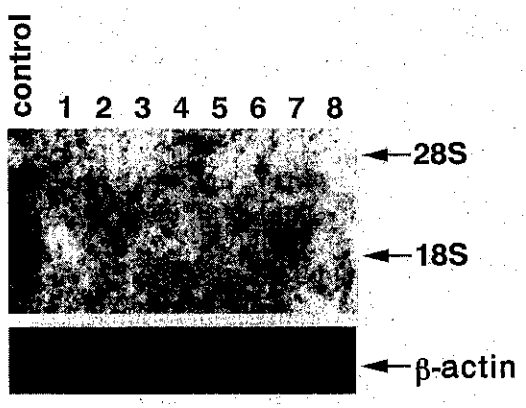

Fig. 2. Northern blot analysis of gastrin receptor mRNA expression in eight colon cancer cell lines. Each lane contains 20 μg of total RNA. Control, human fundic mucosa; lane 1, COLO201; lane 2, COLO205; lane 3, COLO320DM; lane 4, COLO320HSR; lane 5, DLD1; lane 6, HT29; lane 7, LoVo; and lane 8, SW403. β-Actin cDNA was used as a control probe.

bers 3, 4, 5 and 6) (Fig. 4). Interestingly, however, one of the two small cell gastric carcinoma cell lines, ECC10, clearly expressed gastrin receptor gene (Fig. 5).

In order to test for possible alteration of the gastrin receptor gene in ECC10 and LoVo, their cDNA sequences were determined. Several reverse transcriptase-polymerase chain reaction (RT-PCR) products constructed from RNAs extracted from ECC10 and LoVo were sequenced. The nucleotide sequences of the open reading frame of gastrin receptor cDNAs prepared from ECC10 and LoVo were found to be identical with that of human brain.[14]

Next, we examined whether the DNA synthesis of these cells expressing gastrin receptor mRNA is stimu-

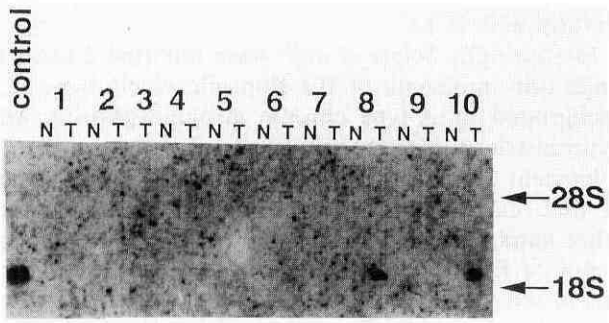

Fig. 3. Gastrin receptor mRNA expression in surgically resected colon cancers. Each lane contains 20 μg of total RNA. Nos. 1 and 2 are cancers of the cecum, No. 3 is a cancer of the transverse colon, and No. 4 is a sigmoid colon cancer. No. 5 to No. 10 are rectal cancers. T stands for tumor tissue and N indicates normal colonic mucosa. No normal tissue was obtained in case No. 3.

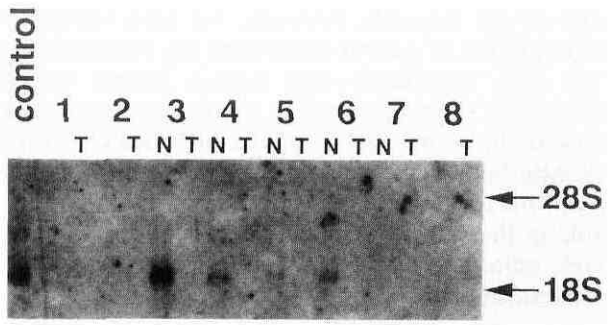

Fig. 4. Gastrin receptor mRNA expression in surgically resected gastric cancers. Each lane contains 20 μg of total RNA. No. 1 to No. 5 are poorly differentiated adenocarcinomas, No. 6 is a moderately differentiated adenocarcinoma, and No. 7 and No. 8 are well differentiated adenocarcinomas. T stands for tumor tissues and N shows corresponding normal gastric mucosa. No normal tissue was obtained in cases 1, 2 and 8.

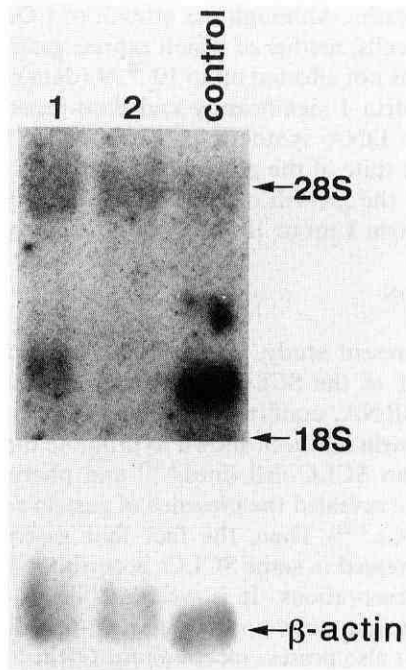

Fig. 5. Gastrin receptor mRNA expression in small cell carcinoma cell lines of the stomach. Each lane contains 20 μg of total RNA. Lane 1, ECC10; lane 2, ECC12; and lane 3, normal fundic mucosa as a positive control. β-Actin cDNA was used as a control probe.

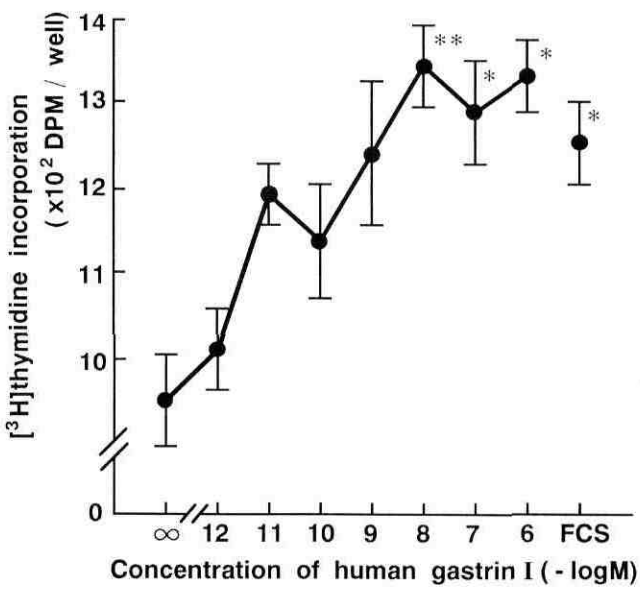

Fig. 6. Effect of human gastrin I on the DNA synthesis of cultured LoVo human colon cancer cell line. Gastrin dose-dependently stimulated [^{3}H]thymidine incorporation into the cells. Vertical lines represent means $\pm$ SE. FCS; 10% fetal calf serum. ** $P<0.01$, * $P<0.05$ vs. control.

lated by gastrin. Although the growth of COLO201 and COLO205 cells, neither of which express gastrin receptor mRNA, was not affected up to 10^{-6} *M* (data not shown), human gastrin I significantly and dose-dependently enhanced the DNA synthesis of LoVo cells (Fig. 6). In contrast, in spite of the presence of gastrin receptor gene expression, the growth of ECC10 was not influenced by human gastrin I up to 10^{-6} *M* (data not shown).

DISCUSSION

In the present study, we demonstrated that approximately half of the SCLC cells tested expressed gastrin receptor mRNA, confirming the recent report by Sethi *et al.*[15] Gastrin has been shown to promote the growth of some human SCLC cell lines,[3, 15] and pharmacological studies have revealed the presence of gastrin receptors on those SCLCs.[3, 22] Thus, the fact that gastrin receptor gene is expressed in some SCLCs accords well with these previous observations. It is well established that many SCLC cells not only produce gastrin-releasing peptide (GRP), but also possess receptors for GRP.[23, 24] Accordingly, GRP is believed to serve as an autocrine growth factor in these SCLC cells.[23] Interestingly, Rehfeld *et al.*[25] have demonstrated the presence of gastrin in some SCLC cells. Therefore, in addition to GRP, gastrin may also act as an autocrine growth factor in SCLC cells.

Our study showed that only one colon cancer cell line, LoVo, and 2 surgically resected colon cancer tissues expressed gastrin receptor mRNA. Although the number of colon cancers which show gastrin receptor gene expression appears to be low, these data seem to be in good agreement with the previous findings that gastrin enhances the growth of some colon cancers.[4, 26] Indeed, in the present study we also found that gastrin significantly enhanced the growth of LoVo cells but did not affect that of COLO201 cells or COLO205 cells, which did not express gastrin receptor mRNA. However, since we were unable to detect any gastrin receptor mRNA expression in normal colonic mucosa, this ectopic expression of gastrin receptor mRNA even in a small number of colon cancers is somewhat surprising. At present we do not have a convincing explanation for it.

Gastrin has also been reported to promote the growth of some gastric cancer cells,[5, 12, 26–28] and indeed, pharmacological studies have demonstrated the presence of gastrin receptors on those carcinoma cells.[3, 5, 12] Therefore, the absence of gastrin receptor gene expression in any of the gastric adenocarcinoma cell lines and gastric adenocarcinoma tissues tested was rather unexpected. However, it should be borne in mind that in normal gastric mucosa, only parietal cells, ECL cells and D cells have been proved to possess gastrin receptors.[13, 29–31] Moreover, poorly differentiated gastric carcinoma is believed to originate from gastric stem cells in the mucous neck proliferative zone,[32] whereas well differentiated gastric carcinoma is considered to be derived from epithelial cells in atrophic mucosa with or without intestinal metaplasia.[33] Thus, whether these progenitor cells possess gastrin receptors or not is an interesting topic for future study.

The most interesting finding in this study was that one small-cell carcinoma cell line of the stomach, ECC10, clearly expressed gastrin receptor mRNA. Although small cell gastric carcinoma is considered to be the gastrointestinal counterpart of SCLC,[34] it is not yet clear whether it has any biological characteristics in common with SCLC. In this regard, the presence of gastrin receptor gene expression in ECC10 observed in this study appears to lend support to the idea that small cell carcinoma of the stomach shares some common biological features with SCLC.

Interestingly, Solcia *et al.*[35] have reported 3 cases of small cell carcinoma of the stomach which arose in a background of A-type chronic atrophic gastritis with hypergastrinemia, suggesting a role of gastrin in the development of small cell gastric carcinoma, as in the case of ECL carcinoid tumor of the stomach.[13, 35] On the other hand, Fujiwara *et al.*[16] observed that the parent tumor of ECC10 had a composite adenocarcinomatous lesion, and suggested that ECC10 developed as a result of a specific process of differentiation from adenocarcinoma cells to endocrine carcinoma cells. Since we failed to demonstrate an enhancement of cellular growth of ECC 10 by gastrin in this study, it is possible that gastrin receptor gene expression in ECC10 has nothing to do with growth, but merely reflects the differentiation of adenocarcinoma toward small cell carcinoma of the stomach.

In summary, the present study has demonstrated that gastrin receptor mRNA is expressed not only in some SCLCs but also in colon cancers and a small cell carcinoma of the stomach. Recently, we have shown that administration of gastrin accelerates the proliferation of CHO cells transfected with human gastrin receptor cDNA.[14] In the present study, we observed that gastrin enhances the growth of LoVo cells, but not ECC10 cells, although both cells express gastrin receptor mRNA. Thus, whether gastrin receptor gene expression may have a role in the growth of those carcinomas or whether it merely reflects some differentiation process is a matter of considerable interest.

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REFERENCES

1) Håkanson, R., Oscarson, J. and Sundler, F. Gastrin and the trophic control of gastric mucosa. *Scand. J. Gastroenterol.*, **12**, 253–256 (1986).
2) Moyer, M. P., Armstrong, A., Aust, J. B., Levine, B. A. and Sirinek, K. R. Effects of gastrin, glutamine, and somatostatin on the *in vitro* growth of normal and malignant human gastric mucosal cells. *Arch. Surg.*, **121**, 285–268 (1986).
3) Sethi, T. and Rozengurt, E. Gastrin stimulates Ca mobilization and clonal growth in small cell lung cancer cells. *Cancer Res.*, **52**, 6031–6035 (1992).
4) Winsett, O. E., Townsend, C. M., Jr., Glass, J. E. and Thompson, J. C. Gastrin stimulates growth of colon cancer. *Surgery*, **99**, 302–307 (1986).
5) Ishizuka, J., Martinez, J., Townsend, C. M., Jr. and Thompson, J. C. The effect of gastrin on growth of human stomach cancer cells. *Ann. Surg.*, **215**, 528–534 (1992).
6) Carlsson, E., Havu, N., Mattsson, H. and Ekman, L. Gastrin and gastric enterochromaffin-like cell carcinoids in the rat. *Digestion*, **47**, 17–23 (1990).
7) Lehy, T., Cadiot, G., Mignon, M., Ruszniewski, P. and Bonfils, S. Influence of multiple endocrine neoplasia type 1 on gastric endocrine cells in patients with the Zollinger-Ellison syndrome. *Gut*, **33**, 1275–1279 (1992).
8) Sjöblom, S.-M., Sipponen, P., Karonen, S.-L. and Jarvinen, K. Argyrophil cell hyperplasia and carcinoid tumors in oxyntic mucosa of stomach. Dependence on duration of pernicious anemia. *Eur. J. Gastroenterol. Hepatol.*, **3**, 153–157 (1991).
9) Weinstoch, J. and Baldwin, G. S. Binding of gastrin-17 to human gastric carcinoma cell lines. *Cancer Res.*, **48**, 932–937 (1988).
10) Upp, J. R., Jr., Singh, P., Townsend, C. M., Jr. and Thompson, J. C. Clinical significance of gastrin receptors in human colon cancers. *Cancer Res.*, **49**, 488–492 (1989).
11) Hoosein, M. N., Kiener, P. A., Curry, R. C. and Brattain, M. G. Evidence for autocrine growth stimulation of cultured colon tumor cell by a gastrin/cholecystokinin-like peptide. *Exp. Cell Res.*, **186**, 15–21 (1990).
12) Watson, S., Durrant, L. and Morris, D. Gastrin: growth enhancing effect on human gastric and colonic tumor cells. *Br. J. Cancer*, **59**, 554–558 (1989).
13) Nakata, H., Matsui, T., Ito, M., Taniguchi, T., Naribayashi, Y., Arima, N., Nakamura, A., Kinoshita, Y., Chihara, K., Hosoda, S. and Chiba, T. Cloning and characterization of gastrin receptor from ECL carcinoid tumor of *Mastomys natalensis*. *Biochem. Biophys. Res. Commun.*, **187**, 1151–1157 (1992).
14) Ito, M., Matsui, T., Taniguchi, T., Tsukamoto, T., Murayama, T., Arima, N., Nakata, H., Chiba, T. and Chihara, K. Functional characterization of a human brain cholecystokinin-B receptor. *J. Biol. Chem.*, **268**, 18300–18305 (1993).
15) Sethi, T., Herget, T., Wu, S. V., Walsh, J. H. and Rozengurt, E. CCK-A and CCK-B receptors are expressed in small cell lung cancer lines and mediate Ca mobilization and clonal growth. *Cancer Res.*, **53**, 5208–5213 (1993).
16) Fujiwara, T., Motoyama, T., Ishihara, N., Watanabe, H., Kumanishi, T., Kato, K., Ichinose, H. and Nagatsu, T. Characterization of four new cell lines derived from small-cell gastrointestinal carcinoma. *Int. J. Cancer*, **54**, 965–971 (1993).
17) Matsui, T., Pierce, J. H., Fleming, J. P. and Greenberger, J. S. Independent expression of human α or β platelet-derived growth factor receptor cDNAs in a native hemopoietic cell leads to functional coupling with mitogenic and chemotactic signaling pathways. *Proc. Natl. Acad. Sci. USA*, **86**, 8314–8318 (1989).
18) Gazdar, A. F., Carny, D. N., Russell, D. K., Sims, H. L., Baylin, S. B., Paul, A., Bunn, J., Guccion, J. G. and Minna, J. D. Establishment of continuous, clonable cultures of small-cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. *Cancer Res.*, **40**, 3502–3507 (1980).
19) Terasaki, T., Shimosato, Y., Nakajima, T., Tsumuraya, M., Hirohashi, S., Yamaguchi, K., Kato, K., Ichinose, H. and Nagatsu, T. Changes in cell characteristics due to culture conditions in cell lines from human small cell lung cancer. *Jpn. J. Clin. Oncol.*, **16**, 203–212 (1986).
20) Yokota, J., Akiyama, T., Fung, Y.-K. T., Benedict, W. F., Namba, Y., Hanaoka, M., Wada, M., Terasaki, T., Shimosato, Y., Sugimura, T. and Terada, M. Altered expression of the retinoblastoma RB gene in small cell carcinoma of the lung. *Oncogene*, **3**, 471–476 (1988).
21) Watson, S. A., Durrant, L. G. and Morris, D. L. Growth-promoting action of gastrin on human colonic and gastric tumor cells cultured *in vitro*. *Br. J. Surg.*, **59**, 554–558 (1989).
22) Staley, J., Jensen, R. T. and Moody, T. W. CCK antagonists interact with CCK-B receptor on human small cell lung cancer cells. *Peptides*, **11**, 1033–1036 (1990).
23) Sausville, E. A., Lebacq-Verheyden, A.-M., Spindel, E. R., Cuttitta, F., Gazdar, A. F. and Battey, J. F. Expression of the gastrin-releasing peptide gene in human small cell lung cancer. *J. Biol. Chem.*, **261**, 2451–2457 (1986).
24) Corjay, M. H., Dobrzamski, D. J., Way, J. M., Viallet, J., Shapira, H., Worland, P., Sausville, E. A. and Battey, J. F. Two distinct bombesin receptor subtypes are expressed and functional in human lung carcinoma cells. *J. Biol. Chem.*, **266**, 18771–18779 (1991).
25) Rehfeld, J. F., Bardrum, L. and Hilsted, L. Gastrin in human bronchogenic carcinomas: constant expression but variable procession of progastrin. *Cancer Res.*, **49**, 2840–2843 (1989).
26) Singh, P., Rae-Venter, B., Townsend, C. M., Jr., Khalil, T. and Thompson, J. C. Gastrin receptors in normal and malignant gastrointestinal mucosa: age-associated

changes. *Am. J. Physiol.*, G761–G769 (1985).
27) Watson, S. A., Durrant, L. G., Crosbie, J. D. and Morris, D. L. The *in vitro* growth response of primary human colorectal and gastric cancer cells to gastrin. *Int. J. Cancer*, **43**, 692–696 (1989).
28) Ochiai, A., Yasui, W. and Tahara, E. Growth-promoting effect of gastrin on human gastric carcinoma cell line TMK-1. *Jpn. J. Cancer Res.*, **76**, 1064–1071 (1985).
29) Chiba, T., Raffoul, K. and Yamada, T. Divergent stimulatory and inhibitory actions of carbamoylcholine on gastric D-cells. *J. Biol. Chem.*, **262**, 8467–8469 (1987).
30) Chiba, T., Fisher, S. K., Park, J., Seguin, E. B., Agranoff, B. W. and Yamada, T. Carbamoylcholine and gastrin induce inositol lipid turnover in canine gastric parietal cells. *Am. J. Physiol.*, **255**, G356–G363 (1988).
31) Prinz, C., Kajimura, M., Scott, D. R., Mercier, F., Helander, H. F. and Sachs, G. Histamine secretion from rat enterochromaffin-like cells. *Gastroenterology*, **105**, 449–461 (1993).
32) Watanabe, H., Hirose, F., Takizawa, S., Terada, Y., Fujii, I. and Ohkita, T. A mode of incipient growth in chemically induced signet ring cell carcinoma of the canine stomach. *Pathol. Res. Pract.*, **164**, 216–223 (1978).
33) Ming, S.-C., Goldman, H. and Reiman, D. G. Intestinal metaplasia and histogenesis of carcinoma in human stomach; light and electron microscopic study. *Cancer*, **20**, 1418–1429 (1967).
34) Jass, J. R., Sobin, L. H. and Watanabe, H. The World Health Organization's histologic classification of gastrointestinal tumors. *Cancer*, **66**, 2162–2167 (1990).
35) Solcia, E., Rindi, G., Fiocca, R., Villani, L., Riv, C. and Capella, C. Endocrine proliferations and carcinoid in hypergastrinemia. *In* "Gastrin," ed. J. H. Walsh, pp. 361–371 (1993). Raven Press, New York.