Characteristics of Cell Lines Established from Human Gastric Carcinoma

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ABSTRACT

We report the establishment and characterization of four continuous cell lines derived from human primary and metastatic gastric carcinomas, and we compare their properties with a panel of colorectal carcinoma cell lines previously established and reported by us. Our success rate in culturing gastric carcinomas was relatively low, especially from primary tumors, compared to colorectal carcinoma. These observations may reflect the relatively modest number of gastric carcinoma cell lines established (mainly from Japan), compared to the abundance of colorectal carcinoma lines established worldwide.

All four gastric lines expressed the surface glycoproteins carcinoembryonic antigen and TAG-72 and three lines expressed CA 19-9. Two of the lines expressed aromatic amino acid decarboxylase but lacked other markers for neuroendocrine differentiation. All four lines were positive for vasoactive intestinal peptide receptors but lacked gastrin receptors. In addition, two lines expressed receptors for muscarinic/cholinergic receptors but not β-adrenergic receptors.

Cytogenetic evidence for gene amplification was present in the cell lines. All four lines contained varying numbers of double-minute chromosomes. One line, SNU-16, was amplified for the c-myc proto-oncogene and contained four homogeneously staining regions. While c-myc and c-erb-B-2 RNA were expressed by all lines, there was no evidence of amplification or overexpression of several other proto-oncogenes and growth factors.

The multiple properties we have described in our gastric carcinoma cell lines are remarkably similar to those found in the panel of colorectal carcinoma cell lines. These properties include morphology, growth characteristics, expression of surface glycoproteins, partial expression of neuroendocrine cell markers, frequent chromosomal evidence of gene amplification, and occasional amplification of the c-myc proto-oncogene. Our four well characterized cell lines should provide useful additions to the modest number currently available for in vitro studies of gastric carcinoma.

INTRODUCTION

Cancer of the stomach is still a leading malignant disease in many countries, including Korea, Japan, eastern Europe, Iceland, and South Africa (1, 2). While the incidence in the United States is declining, stomach cancer is the sixth most common cause of cancer deaths and still remains a major health problem (3). Thus far, the only treatment that results in significant cure of gastric cancer is well planned, aggressive, subtotal or total gastrectomy (4). Because one third of patients will present with unresectable disease and another one third of patients will develop local or distant recurrence following curative surgery, other modalities of therapy have been studied in attempts to improve treatment of widespread cancer and to prevent relapse in patients with resectable disease.
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Chromosome slides were prepared by the standard air-dried method from exponentially growing cultures, after colcemid treatment at approximately 0.1 μg/ml final concentration for 45 min. They were stained with the fast G-banding (20) for an overall karyotypic analysis and with Q- and C-banding (21) to obtain complementary evidence for the identification of specific chromosomes and chromosome segments. Chromosome number distribution was evaluated from 50 metaphases, and the rate of higher ploidyies (as compared with the modal chromosome number) was estimated from 500 metaphases.

Aromatic amino acid decarboxylase (EC 4.1.1.28), also known as L-DOPA decarboxylase, was assayed as previously described (22, 23). Values are reported in units (1 unit = 1 nmol CO2 released/h/mg soluble protein).

All cell lines were immunopositive by indirect immunofluorescence and flow microfluorometry, using the FACS II flow cytometer (Becton Dickinson, Mountain View, CA) equipped with an argon laser, utilizing the 488-nm line at 0.5-mW power. Monclonal antibodies used were B1 (CD20, pan-B-cell; Coulter, Hialeah, FL), B2 (CD21, CR2 receptor; Coulter), B4 (CD19, pan-B; Coulter), T11 (CD2, pan-T cell; Ortho, Raritan, NJ), Leu-9 (CD7, pan-T-cell; Becton Dickinson), T9 (transferrin receptor; Ortho), and the natural killer cell markers Leu-7, Leu-15 (CD11b) (Becton Dickinson), and NKH1 (Coulter). A viable single-cell suspension was prepared by incubation of the cells with a mixture of collagenase I (140 mg/100 ml; Calbiochem, La Jolla, CA) and DNAse I (30 mg/100 ml; Calbiochem) in phosphate-buffered saline, for 30 min at 37°C, with gentle stirring. The cells were washed in saline and resuspended at a concentration of 10^6/ml in phosphate-buffered saline with 1% bovine serum albumin and 0.1% sodium azide (PBA), for incubation with primary antibody, using 0.5 × 10^6 cells/ marker. Following incubation for 30 min with primary antibody, the cells were washed with PBA, incubated with fluorescein-conjugated goat anti-mouse immunoglobulin (Coulter) for 30 min at 4°C, washed, and resuspended in PBA for analysis.

Mycoplasma contamination was tested for by direct culture in agar, the Hoechst stain method, and the use of a ribosomal RNA hybridization method (Gen-Probe, San Diego, CA). All lines were tested for murine virus contamination by the mouse antibody production tests (24). Cell homogenates were tested for the human forms of the following enzymes by starch gel (25), using the Authentikit system (Corning Science Products, East Walpole, MA): purine nucleoside phosphorylase (EC 2.4.2.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), peptidase B (EC 3.4.11.4), and lactate dehydrogenase (EC 1.1.1.27).

Antigen Expression and Secretion. Expression and secretion of CEA2 (26, 27), CA 19-9 (28), and TAG-72 (29, 30) surface antigens were determined in an Auto-Gamma 5000 Series gamma counter (Packard Instrument Corp., Sterling, VA). When tritiated radioligand was used, incubation with iodinated radioligand, 0.133 ml of cells were sampled, and DNA was prepared (36), digested in a 1% agarose-formaldehyde gel and transferred to nylon filters (Nytran). The probes used were labeled with 32P using nick translation.

Detection of Surface Receptors by Radioligand Binding. 125I-BH-CKK-8 (2200 Ci/mmol), 125I-BH-SP (2200 Ci/mmol), 125I-gastrin-17 (2200 Ci/mmol), 125I-VIP (2200 Ci/mmol), 125I-OH-BZP (2200 Ci/mmol), [3H]-NMS (70-87 Ci/mmol), and tyrosyl-[3,5-3H]enkephalin (30-50 Ci/mmol) were obtained from New England Nuclear (Boston, MA). 125I-Tyr^4-bombesin (2200 Ci/mmol), 125I-BH-NMB (2200 Ci/mmol), and 125I-CGRP (2200 Ci/mmol) were prepared using the methods described previously (37-39). Cholecystokinin octapeptide was obtained from Research Products International Corp. (Raritan, NJ); Tyr^4-bombesin was obtained from Bachem Inc. (Torrance, CA); gastrin-I, neuroimunogen B, VIP, secretin, substance P, and CGRP were obtained from Peninsula Laboratories (Belmont, CA); pindolol, naloxone hydrochloride, and atropine were obtained from Sigma (St. Louis, MO).

Cells were washed and resuspended in 50 mm Tris buffer, consisting of 130 mm NaCl, 7.7 mm KCl, 5 mm MgCl2, 0.1% bovine serum albumin, 1 mm ethyleneglycol bis-(β-aminoethyl ether)-N,N,N,N'-tetraacetic acid, 0.1% bacitracin, 4 ng/ml leupeptin, and 2 mg/ml soybean trypsin inhibitor. Cells at a concentration of 15 × 10^6 cells/ml (range, 5–25 × 10^6 cells/ml), in a volume of 0.5 ml were incubated with radioligand at a concentration of 50 pm for 125I-BH-CKK-8, 125I-Tyr^4-bombesin, 125I-BH-NMB, 125I-VIP, and 125I-OH-BZP; 100 pm for 125I-gastrin-17; 125 pm for 125I-BH-SP and 125I-CGRP; 0.6 nm for [3H]-NMS; and 1 nm for tyrosyl-[3H]enkephalin, for 30 min at 22°C. After incubation with iodinated radioligand, 0.133 ml of cells were sampled, spun down through cell buffer containing 4% bovine serum albumin in a Beckman Microfuge, and then washed twice, and the pellet was counted in an Auto-Gamma 5000 Series gamma counter (Packard Instrument Corp., Sterling, VA). When tritiated radioligand was used, cells were sampled, washed over glass microfiber filters (Whatman International Ltd., Maidstone, England), and then counted in a liquid scintillation spectrometer (Packard Instruments Corp., Sterling, VA).

Non saturable binding was the amount of radioactivity associated with the cells when the incubation contained radioligand plus the unlabeled peptide in concentrations of 1–10 μM.

RESULTS

Establishment of Cell Lines. We attempted to culture 30 primary and 12 metastatic gastric carcinomas. With one exception (see below), all specimens were from Korean patients. Four gastric carcinoma cell lines were established from one primary and three metastatic tumors. Thus, our overall success rates

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were 3% from primary tumors and 25% from metastatic tumors.

One cell line, NCI-N87, was established from a liver metastasis of a gastric carcinoma arising in an American patient. It was passaged as a xenograft in athymic nude mice for three passages before establishing it as a cell line. The remaining lines were established from Korean patients (Table 1). Three of the lines were from patients who had not received prior cytotoxic therapy, while one line was from a patient who had previously received chemotherapy including 5-fluorouracil, doxorubicin, and mitomycin-C.

Cell Line Characteristics. Cell line NCI-N87 grew as an adherent monolayer consisting of tightly knit epithelial cells (Table 2, Fig. 1). The other three cultures displayed both adherent and floating subpopulations (Table 1), and the cells appeared more rounded (Fig. 1).

Cell lines NCI-N87 and SNU-16 were tumorigenic in athymic nude mice (the other two lines were not tested). Population doubling times ranged from 26 to 47 h. Cloning efficiencies ranged from 1.9 to 27% (Table 2). All lines expressed human forms of the four enzymes tested and were free of contamination with Mycoplasma or murine viruses.

Based on culture, xenograft, and ultrastructural morphology, cell line NCI-N87 was classified as well differentiated and the other three lines as poorly differentiated (Table 2, Figs. 1 and 2). These morphologies were identical to the degree of differentiation of the original tumor. However, one poorly differentiated cell line (SNU-16) formed small numbers of goblet cells in culture (Fig. 2D).

Expression and Secretion of Antigens. We studied expression and secretion of antigens associated with gastrointestinal cells and their tumors. As displayed in Fig. 3, all three antigens were found in cell pellets of the gastric lines at levels comparable to those present in colorectal carcinoma lines, although the levels of CA 19-9 demonstrated considerable variation between gastric cell lines. Except for TAG-72, which is not generally secreted, the antigens were also detected in supernatant fluids (data not shown). Only one gastric carcinoma cell line (SNU-1) had detectable concentrations of TAG-72 antigen in its supernatant fluid (900 ng/10^6 cells). With colorectal cell lines, there was no correlation between degree of morphological expression by the gastric lines and antigen concentrations.

NE Cell Features. We assayed for DDC enzymatic activity, an enzyme characteristic of NE cells. Relatively high levels of enzyme activity (87–89 units/mg protein) were detected in two lines (SNU-5 and SNU-16), both initiated from ascitic fluids. However, expression of neuroendocrine markers by these lines was partial, because all lines lacked other evidence of neuroendocrine differentiation (dense core granules, chromogranin A RNA, and the natural killer cell antigens Leu-7 and NKH1).

Receptor Binding Studies. Saturable binding of 125I-VIP was seen in SNU-1, SNU-5, and SNU-16 but was minimally detectable in NCI-N87 (Table 3). With 50 pm 125I-VIP, the saturable binding as a percent of the total binding was 8, 54, and 36% in SNU-1, SNU-5, and SNU-16, respectively. A small but appreciable amount of saturable binding of [3H]-NMS was seen in SNU-5 and NCI-N87 but not in SNU-1 or SNU-16 (Table 3). With 0.6 nm [3H]-NMS, the saturable binding as a percent of the total binding was 26% and 29% in cell lines SNU-5 and NCI-N87, respectively. There was no saturable binding of the other radioligands (Table 3).

To further examine the interaction of 125I-VIP with receptors on one of these cell lines, SNU-1, we studied the ability of VIP and secretin to inhibit binding of 125I-VIP. VIP caused detectable inhibition at 0.3 nm, half-maximal inhibition at 6.3 nm, and complete inhibition at 0.3 μM (Fig. 4). Secretin, although structurally related to VIP, failed to inhibit binding of 125I-VIP at concentrations of 0.1 μM, demonstrating it was at least 3000 times less potent than VIP and that 125I-VIP was in fact binding to a VIP receptor and not to a secretin receptor. When the data were analyzed using a nonlinear least squares curve-fitting program, LIGAND (40), the inhibition of binding of 125I-VIP by VIP was best fit with a one-receptor model having a high affinity for VIP (Kd = 2.5 nm).

Gene Amplification and Expression. In one gastric cell line, SNU-16, an intense signal corresponding to the 12.7-kilobase EcoRI c-myc fragment was detected (Fig. 5). We estimated that the signal from this line was about 50-fold greater than the germ line level. No evidence of amplification or rearrangements was noted with the N-myc, L-myc, myb, and EGF receptor genes.

All of the gastric cell lines expressed levels of c-myc and erbB RNA that were comparable to the levels detected in a panel of 11 colorectal carcinoma cell lines (data not shown). Of interest, the level of c-myc RNA expressed by the SNU-16 cell line was comparable to those expressed by the other three lines. No expression of the following genes by any of the cell lines was detected: N-myc, L-myc, sis, IGF-2, and gastrin releasing peptide.

Cytogenetic Studies. Each cell line had unique modal karyotypic characteristics, indicating its independent origin. Numerical data on the chromosome characteristics of four cell lines are summarized in Table 4. SNU-1 and NCI-N87 had near-diploid and the other two lines had near-tetraploid modal chromosome numbers. Of interest, both near-tetraploid lines were initiated from malignant ascites fluids. The rate of cells with higher ploidy, as compared with the modal stem line chromosome numbers, was high in all but SNU-16. Although the bimodal distribution within the modal chromosome number ranges (i.e., excluding the higher ploidy) was obvious only in SNU-16, two distinct sublines (in each cell line) could be identified by chromosome band analysis (Fig. 6). Each subline had a unique and consistent subset of chromosomes that distinguished it. Generally, these differences totaled less than 10% of the chromosome complements. DMs were found in 12–64% of the cells of all four cell lines (Table 4). However, only one or two DMs/cell were seen in all lines except SNU-16, and

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Table 1 Origin of gastric carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Date of initiation</th>
<th>Age</th>
<th>Country of origin</th>
<th>Blood type</th>
<th>Prior therapy</th>
<th>Tumor site*</th>
<th>Original tumor differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-N87</td>
<td>8/76</td>
<td>M</td>
<td>USA</td>
<td>?</td>
<td>None</td>
<td>Liver</td>
<td>Well</td>
</tr>
<tr>
<td>SNU-1</td>
<td>4/84</td>
<td>44</td>
<td>Korea</td>
<td>0</td>
<td>None</td>
<td>Stomach</td>
<td>Poor</td>
</tr>
<tr>
<td>SNU-5</td>
<td>6/87</td>
<td>33</td>
<td>Korea</td>
<td>0</td>
<td>FAM*</td>
<td>Ascies</td>
<td>Poor</td>
</tr>
<tr>
<td>SNU-16</td>
<td>7/87</td>
<td>33</td>
<td>Korea</td>
<td>A+</td>
<td>None</td>
<td>Ascies</td>
<td>Poor</td>
</tr>
</tbody>
</table>

* Tumor site used for cell culture.
FAM, 5-fluorouracil, doxorubicin, and methotrexate.

Table 2 Culture morphology and growth characteristics of gastric cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Substrate adherence</th>
<th>Differentiation</th>
<th>Doubling time (h)</th>
<th>Cloning efficiency</th>
<th>Tumorigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-N87</td>
<td>A</td>
<td>Well</td>
<td>47</td>
<td>4.3</td>
<td>+</td>
</tr>
<tr>
<td>SNU-1</td>
<td>F/A</td>
<td>Poor</td>
<td>26</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>SNU-5</td>
<td>F/A</td>
<td>Poor</td>
<td>34</td>
<td>27</td>
<td>NT*</td>
</tr>
<tr>
<td>SNU-16</td>
<td>F/A</td>
<td>Poor</td>
<td>27</td>
<td>10</td>
<td>NT</td>
</tr>
</tbody>
</table>

* A, adherent; F/A, floating and adherent subpopulations.
* The partially floating SNU cell lines were tested in semisolid medium, while the plating efficiency of the adherent cell line (NCI-N87) was determined.
* NT, not tested.
only a portion of cells in all lines possessed this aberration. In SNU-16, 4 to over 20 DMs were detected in some cells in both sublines (Fig. 6).

HSRs were present in the minor subline of SNU-16. This subline had HSRs occurring at four separate chromosomal locations (6q, 11p, and two unidentified marker chromosomes). Up to three HSRs were present in individual cells (Fig. 6). The 11p HSR was the most common of the HSR chromosomes, whereas 9q HSR occurred only once. A more detailed account of the HSRs will be reported elsewhere.

DISCUSSION

In this report we describe the establishment and characterization of four continuous cell lines derived from human primary and metastatic gastric carcinomas. Our success rate was relatively low for gastric carcinomas, especially from primary tumors, compared to our success with colorectal carcinomas (about 40% from both primary and metastatic tumors) (6).3

These findings reflect the relative paucity of gastric carcinoma lines established worldwide, compared to the abundance of colorectal carcinoma lines. Of interest, we have recently reported that the success rate of culturing non-small cell carcinomas is much higher with metastatic tumors than with primary tumors and that successful culture may be a negative prognostic factor (41).

While a modest number of gastric carcinoma lines have been initiated by other investigators, mostly from Japan, our cell lines express several interesting or unique features, including (a) expression and secretion of three gastrointestinal cell associated antigens; (b) frequent expression of the NE cell marker DDC; and (c) expression of vasoactive peptide and muscarinic/cholinergic receptors. We have also characterized a possibly unique cell line with c-myc gene amplification and having a subline with HSRs at four separate chromosome locations. Because we are not aware of any comprehensive review of the properties of gastric carcinoma cell lines established from Oriental or Western patients, we cannot directly compare the characteristics of our panel of cell lines with those from other sources.

We investigated production and secretion of three membrane-bound glycoproteins associated with gastrointestinal cells (26-30). CEA is present on normal and malignant colonic tissues as well as other cancers. CA 19-9, originally identified from a colon carcinoma line, is a sialylated lacto-N-fucopentaose II, an oligosaccharide related to Lewis blood group substances. TAG-72, a high molecular weight glycoprotein, is expressed by many epithelial malignancies including colon, breast, and ovary. Its expression appears to be influenced by the spatial configuration of the tumor cells, and it is reported to be rarely expressed in monolayer cultures. Of interest, all our gastric lines expressed TAG-72 antigen, although the concentration was lowest in the well differentiated NCI-N87 cell line demonstrating the greatest degree of substrate adherence. All four lines also expressed

3 A. F. Gazdar, J-G. Park, and H. Oie, unpublished data.
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Fig. 2. Ultrastructural morphology of gastric carcinoma cell lines. A, well differentiated cell line NCI-N87 (× 3000). Tight junctions (arrows) and short microvilli are present on the free surfaces. Lysosomal granules are present in the cytoplasm. These features are suggestive of the intestinal type of gastric carcinoma. B–D, poorly differentiated cell lines SNU-1 (B) (× 3500), SNU-5 (C) (× 6000), and SNU-16 (D) (× 8000). The cells of these lines are loosely coherent and contain microvilli which vary in number and length. Bundles of intermediate filaments are present in SNU-5. While most of the cells of SNU-16 were relatively undifferentiated, varying amounts of mucin were present. Occasional cells demonstrated a goblet cell morphology (as illustrated), with eccentric nuclei and cytoplasm filled with mucin droplets.

CEA, and three expressed CA 19-9. Lines expressing CEA and CA 19-9 actively secreted these antigens into the supernatant fluids, while only modest concentrations of TAG-72 antigen were present in the supernatant fluids of one cell line. TAG-72 is usually not secreted by expressor cells, while the other two antigens are usually actively secreted. As previously reported (6) and displayed herein, the results of secretion of these antigens by the colorectal cell panel were similar, although the levels of TAG-72 expression in three of four gastric lines were higher than in the colorectal lines.

Two of our gastric cell lines expressed varying concentrations of DDC, a key NE cell marker (42). DDC is essential for formation of biogenic amines, both serotonin and catecholamines. But other NE cell markers (dense core granules, chromogranin A, Leu-7, and NKH1 antigens) were not expressed. These findings suggest that gastric tumors, as with colorectal carcinomas, may frequently express part of the program of NE cell differentiation (6, 23). Because three of our four patients from whom cell lines were established were not treated with systemic chemotherapy, we cannot determine whether NE differentiation affects the response to chemotherapy or survival.

As with our colorectal cell lines (6), DMs appeared to occur frequently in stomach lines. In two of the gastric lines, the appearance of DMs was obvious, since they were either present in abundance (NCI-N87) or present in the majority of cells (SNU-16). In the other two lines, only a single DM was detected in a minority of the cells. DM-like artifacts may occur in some instances (43). Thus, our identification of DMs in these two cases needs to be interpreted with caution, although we believe our observations to be correct, based on examination of at least 50 metaphases photographed for karyotypic analysis.

The presence of both DMs and HSRs or only DMs in some cells from cell line SNU-16 are of special interest. The colorectal carcinoma line, COLO 320 (44), from which the current COLO 320DM and COLO 320HSR sublines were isolated (44), probably had a similar situation (i.e., tumors with DMs evolved into culture sublines having one or more HSRs). The presence of only DMs or HSRs in different sublines of the same
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Fig. 3. Antigen expression in gastric and colorectal cell lines. The relative concentrations of CEA, CA 19-9, and TAG-72 present in cell pellets of gastric and colorectal carcinoma cell lines are illustrated on a logarithmic scale.

Table 3 Binding of radiolabeled peptides to human gastric carcinoma cell lines

<table>
<thead>
<tr>
<th>Radiolabeled peptide added</th>
<th>NCI-N87</th>
<th>SNU-1</th>
<th>SNU-5</th>
<th>SNU-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>113I-VIP</td>
<td>9.4</td>
<td>168</td>
<td>29</td>
<td>61</td>
</tr>
<tr>
<td>113I-BH-SP</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>113I-BH-CCK 8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>113I-Gastrin-17-1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>113I-Tyr4-bombesin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>113I-Neureomedin B</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>123I-CGRP</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>123I-OH-BZP</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>[3H]NMS</td>
<td>85</td>
<td>ND</td>
<td>67</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detected.

Table 4 Summary of cytogenetic data of the gastric carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Modal chromosome no.</th>
<th>% of cells with modal number Ranges</th>
<th>% of cells with higher ploidies</th>
<th>% DM</th>
<th>% HSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-N87</td>
<td>43</td>
<td>39</td>
<td>38-45</td>
<td>16.6</td>
<td>64</td>
</tr>
<tr>
<td>SNU-1</td>
<td>47</td>
<td>86</td>
<td>85-98</td>
<td>5.2</td>
<td>28</td>
</tr>
<tr>
<td>SNU-5</td>
<td>89</td>
<td>36</td>
<td>85-98</td>
<td>9.6</td>
<td>16</td>
</tr>
<tr>
<td>SNU-16*</td>
<td>92</td>
<td>16</td>
<td>81-94</td>
<td>1.0</td>
<td>12</td>
</tr>
</tbody>
</table>

* In addition, SNU-16 contained two subpopulations with modal chromosome numbers of 86 and 92 (see text).

Fig. 4. Specific binding of 113I-VIP to SNU-1 gastric carcinoma cells in the presence of varying concentrations of unlabeled VIP and secretin as shown.

Fig. 5. Hybridization comparison of EcoRI digests of gastric and small cell lung (SCLC) carcinoma cell line DNA with human c-myc probe. The position of the germ line (not shown) and all cell line c-myc genes is 12.7 kilobases. The DNAs of small cell lung cancer line NCI-N417 and gastric line SNU-16 show approximately 50-fold amplification of the c-myc germ line, relative to the other cell lines.

cell line suggests a probable causal relationship between these aberrations. Of interest, the c-myc proto-oncogene is amplified in both COLO 320 and SNU-16 cell lines.

All four human gastric cell lines studied were positive for VIP receptors and two were positive for muscarinic/cholinergic receptors in low numbers. These results are in agreement with prior studies which have revealed the presence of VIP receptors on occasional human cell lines of both gastric and colonic origin (45, 46). Although gastrin receptors have also been described on human cell lines of gastric and colonic origin (47, 48) we were unable to demonstrate gastrin binding to the four cell lines studied here. This may be due to differences in methods of cell growth or binding studies but is more likely due to the heterogeneity of gastric neoplasms. The evidence that peptide receptors exist on these cell lines suggests the possibility that growth and signal transduction may be modulated by either receptor agonists or antagonists, as has been shown for gastrin receptors on colon carcinoma cells and VIP receptors on gastric carcinoma cells (45, 47).

Of interest, we found two gastric cell lines, one differentiated and one poorly differentiated, that expressed receptors for muscarinic cholinergic agents. Receptors of this type have been found in pancreatic acini, where they play a role in amylase release (49). However, to the best of our knowledge, they have not been described previously on gastric carcinoma cells. We did not detect evidence for expression of several other peptide receptors or β-adrenergic receptors on our gastric carcinoma cell lines.

The myc genes are proto-oncogenes frequently associated with cytogenetic evidence of gene amplification (32, 50). One gastric line, SNU-16, showed c-myc gene amplification, HSRs, and DMs. Similar findings were present in colorectal cell lines NCI-H716 (6) and COLO 320 (44). Previously, c-myc gene amplification in human gastric cancers has only been reported in xenografts transplanted into nude mice (51, 52). All of the gastric lines, including line SNU-16, expressed relatively low

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levels of c-myc RNA. Other oncogenes occasionally described to be amplified or overexpressed in gastric tumors and cell lines include c-erb-B-2 (53), transforming gene hst (also known as HSTF1) (54, 55), and the ras family (56–58).

The multiple properties we have described in gastric carcinoma cell lines are remarkably similar to those found in a panel of colorectal carcinoma cell lines initiated by us (6). These properties include morphology, growth characteristics, expression of surface glycoproteins, partial expression of NE cell markers, frequent chromosomal evidence of gene amplification, and occasional amplification of the c-myc proto-oncogene. However, clinical studies indicate that there are major differences in the biology of these two tumor types. The in vitro chemosensitivity patterns to cytotoxic drugs and expression of the multidrug resistance-associated (MDR1) gene are very different in the gastric and colorectal cell lines (59). In addition, colorectal carcinoma cell lines express receptors for peptide hormones more frequently than gastric carcinoma cell lines (60).

Our four well characterized cell lines should provide useful additions to the modest number currently available for the in vitro study of gastric carcinoma.

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