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Biology of Colorectal and Gastric Cancer Cell Lines

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Abstract Cell lines established from the human colorectal and gastric cancers may provide very useful tools to the study of the disease and to develop and test new therapeutic approaches, and a large bank of well-characterized cell lines should reflect the diversity of tumor phenotypes and provide adequate models for the study of tumor heterogeneity. Colorectal lines are relatively easy to establish, while gastric cancer cell lines remain extremely difficult to propagate in long-term culture, and the number of cell lines is very limited. In this paper, we describe the up-to-date results of the characteristics of our nine colorectal cancer cell lines and four gastric cancer cell lines. Based on culture, xenograft, and ultrastructural morphologies, these cell lines could be subtyped into well-differentiated, moderately differentiated, poorly differentiated, and mucinous carcinomas. Basic properties concerning expression and secretion of antigens, neuroendocrine features, receptor binding of various gastrointestinal hormones and neurotransmitters, cytogenetic studies, gene amplification and expression, and chemosensitivity profiles are described. In particular, a greater number of receptors for hormones and neurotransmitters are expressed on human colorectal cancer cell lines compared to gastric cancer cell lines, raising the possibility that gastrointestinal hormones may have a greater autocrine effect on colon cancer cell growth. Despite major differences in the biology of colorectal cancer and gastric cancer as indicated by clinical studies, the multiple properties that we examined reveals marked similarities between the colorectal and gastric cancer cell lines. However, in vitro chemosensitivity patterns to cytotoxic drugs are very different in colorectal and gastric cell lines. Some of these observations may be due to the relatively low expression of the multidrug-resistance-associated (MDR1) gene in gastric cancer cell lines. In addition, colorectal cancer cell lines express receptors for peptide hormones more frequently.

Colorectal cancer is one of the most common solid tumors, and its incidence is second only to lung cancer in the United States. An estimated 138,000 new cases will have been diagnosed in 1995, 55,000 of whom will die of their disease [1].

And in the case of gastric cancer, although the incidence has been continuously and markedly decreased in most western countries for the past 40 years, it is still high in Korea, Japan, China, Russia, Finland, and Chile [2,3]. In Korea, the frequency of gastric cancer among all admitted malignancy patients is 24.0% (29.2% in males and 18.3% in females) [2], and the gastric cancer deaths were the highest in both males and females (32 gastric cancer deaths per 100,000 population). In the United States, although the incidence of gastric cancer is declining, gastric cancer a major cause of cancer deaths and still remains a major health problem [1,4].

In the establishment of cell lines from carcinomas, gastric carcinoma has been much more difficult to culture than colorectal carcinomas. While over 80 cell lines established from colorectal carcinoma exist [5–7], only a modest number of gastric carcinoma lines have been established [8].

Any in-depth study requires a comprehensive panel of well-characterized cell lines. A large bank of well-characterized cell lines may reflect the diversity of tumor phenotypes and provide adequate models for studying tumor heterogeneity.

In this article, we describe the establishment and characterization of four gastric carcinoma cell lines, three from Korean patients and one

Abbreviations used: CEA, cacinoembryonic antigen; DDC, L-dopa decarboxylase; NE, neuroendocrine; VIP, vasoactive intestinal peptide; NMS, N-methylscopolamine; DM, double minute; HSR, homogeneously staining region; EGF, epidermal growth factor; IGF, insulin-like growth factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (tetrazolyl blue); 5-FU, 5-fluorouracil; AUC, area under the curve; LV, leucovorin; SCLC, small cell lung cancer.

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from an American, and 9 colorectal carcinomas, four from Korean and five from American patients.

ESTABLISHMENT OF CELL LINES

Some human tumors, such as melanoma, are relatively easy to culture. Others, such as pheochromocytomas and islet cell carcinomas, are exceedingly difficult, and no established cell lines currently exist. Rutzky and Moyer [9] summarized key features relevant to a successful culture: (1) nonenzymatic or minimal dissociation of tumor tissue, (2) seeding cultures as explant and at high cell densities, (3) removal of contaminating fibroblasts usually after they have aided cultural initiation, and (4) delaying passage until high cell densities have been achieved and plating cells at high density (both of these procedures increase concentrations of conditioning or paracrine factors).

Details of tumor disaggregation, culture methods and growth media have been published previously [see references 13,14]. Solid tumors were finely minced with scissors and dissociated into small aggregates by pipetting. For culture of primary tumors, invasive areas were selected from the serosal surface, whenever possible, to decrease the chance of microbial contamination. Ascitic fluids were collected, pelleted, washed, and resuspended in growth medium. Approximately 1–5 $\times$ 10^6 cells were seeded into 25 cm^2 flasks.

In establishment of gastric carcinoma cell lines, tumors were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (R10). In the case of colorectal carcinoma cell lines, tumors were cultured in ACL-4, the medium were formulated for bronchial adenocarcinomas [10,11].

ACL-4 is a complex medium, consisting of 12 additives to basal medium. Four of the five HITES (medium for the selective growth of SCLC; the components of HITES included insulin, transferrin, hydrocortisone, estradiol, and selenium added to a basal medium, RPMI-1640) additives are present in ACL-4; it lacks only estradiol. Epidermal growth factor (EGF) is also present. Bovine serum albumin helps to compensate for some of the high molecular weight proteins present in serum, and aids growth in semisolid media. In addition, we added triiodothyronine (a selective mitogen), ethanolamine, and phosphorylethanolamine (precursors of membrane lipids), HEPES buffer (to compensate for the loss of buffering properties of serum), glutamine, and sodium pyruvate (added empirically).

Initially, cultures were passaged whenever vigorous tumor cell growth was observed. Subsequent passages were performed weekly. Nonadherent cultras were passed by transfer or floating multicellular aggregates. Adherent cultures were passaged at subconfluence after trypsinization [12] was used to obtain a pure tumor cell population. Media and sera were obtained from Grand Island Biological Co. (Grand Island, NY). Cultures were maintained in humidified incubators at 37°C in an atmosphere of 5% CO_2 and 95% air.

CULTURE CHARACTERISTICS

Here, we selected 9 colorectal carcinoma cell lines [13] and 4 gastric carcinoma cell lines [14], for detailed characteristics (Tables I, II). Seven (SNU-C1, SNU-C2A, SNU-C4, SNU-C5, SNU-1, SNU-5, SNU-16) of the lines were originated from Korean patients in Korea. Three lines (SNU-C2A, SNU-C4, SNU-C5) were cultured from xenografts in athymic nude mice, and the remainder were cultured directly from tumor material. Cell lines NCI-N87 and SNU-1 were tumorigenic in athymic nude mice. Population doubling time ranged from 27 to 93 h. Cloning efficiencies ranged from 1.9 to 27%.

MORPHOLOGIC CHARACTERISTICS

Most colorectal carcinoma cell lines demonstrated substrate adherence in serum supplemented media (SSM) and lacked it in the ACL-4 medium unless the culture dishes were precoated with collagen. In contrast, three of four gastric carcinoma cell lines grew as floating cells.

Illustrations of the culture characteristics of colorectal and gastric cell lines have been published previously [13,14]. In R10 medium, one culture, SNU-C1, displayed both adherent and floating subpopulations. Cell line NCI-H498 grew as floating cell aggregates surrounded by a halo of easily visualized mucinous material. If the mucin coat was removed and the cells dispersed by trypsinization, the line could be adapted into monolayer cultures. Cell line NCI-H716 completely lacked substrate adherence and grew as amorphous floating cell aggregates in R10 medium. In ACL-4, which lacks attachment factors, 7 of 9 colorectal carcinoma lines lacked substrate adherence and grew as floating gland-like structures or amorphous aggregates. Most of the floating cultures could be grown as mono-

layers in ACL-4 medium if the culture dishes were precoated with collagen.

Evidence of glandular differentiation was present in 5 colorectal cultures. In SSM, 2 of the 9 colorectal lines demonstrated a prominent dome formation [15]. Culture morphology was most accurately determined in ACL-4 medium, although some features could also be discerned in R10 medium. Dome formation could only be evaluated in confluent monolayers grown in R10 medium. One gland- and dome-forming culture, NCI-H498, secreted large amounts of extracellular mucin. This feature was best appreciated in floating cultures, where cellular aggregates were surrounded by halo of extracellular mucin, which could readily be seen by phase microscopy. Based on culture, xenograft, and ultrastructural morphologies, the lines could be divided into 4 subtypes.

Well Differentiated Cell Lines (NCI-H548, NCI-H630, NCI-N87)

Cell line NCI-N87 grew as an adherent monolayer consisting of tightly knit epithelial cells. NCI-H548 and NCI-H630 cell lines grew as floating cell aggregates, sometimes as acinar structures surrounding central laminae in ACL-4 medium. A characteristic feature of two cell lines was the uniform, radial orientation of tall columnar cells. In R10 medium they consisted of relatively large epithelioid cells. Prominent dome formation was present at confluence two of the cell lines. In addition to dome formation, three dimensional gland-like structures were present in confluent adherent cultures. The ultrastructural appearances of gland-forming cultures demonstrated features of colonic mucosa, including uniform microvilli with prominent filamentous core rootlets, glycocalyceal bodies, and well-formed junctional complexes that were present between cells lining glands. Xenografts of well-differentiated cultures reflected the features of their respective cultures. Well-formed glands were lined by tall columnar cells with prominent apical borders.

Moderately Differentiated Cell Lines (NCI-H508, SNU-C1)

SNU-C1 displayed both adherent and floating subpopulations in R10 medium. In ACL-4 medium, the cells formed spheroidal or irregular masses rather than gland-like structures. In R10 medium, dome formation, if present, was inconspicuous. The cells tended to be cuboidal rather than columnar, and gland formation was less prominent. Microvilli were less uniform in size, and filamentous core rootlets, glycocalyceal bodies, and well-formed junctional complexes were only present occasionally. Xenografts of moderately differentiated cultures had glands that were less well organized; they were lined by cuboidal cells and varied considerably in size.

Poorly Differentiated Cell Lines (SNU-C2A, SNU-C4, SNU-C5, NCI-H716, SNU-1, SNU-5, SNU-16)

In ACL-4, 4 colorectal lines, SNU-C2A, SNU-C4, SNU-C5, and NCI-H716, grew as single cells or small amorphous aggregates. In R10 medium, they displayed varying degrees of substrate adherence, but cell line, NCI-H716, did not attach at all. No evidence of gland or dome formation was observed. These lines grew as sheets of attached epithelial cells lacking dome formation and other distinguishing characteristics, or as amorphous floating cell aggregates lacking central lumina. Abrupt shifting of medium for SNU-C5 to serum free simple RPMI was possible. This characteristic might be useful for studying autocrine factors.

Ultrastructural studies confirmed the epithelial nature of these lines (microvilli, desmosomes) and demonstrated occasional attempts to form inter- and intracellular glands. No characteristic ultrastructural features were seen, except in cell line NCI-H716. The undifferentiated cell line, NCI-H716, demonstrated a unique ultrastructural feature, the presence of intracellular dense core granules. The granules, which were approximately 300 nm in size, were bound by a unit membrane and contained electron-dense cores surrounded by an electrolucent halo. These structures are characteristic of neuroendocrine (NE) cells and tumors. Xenografts usually demonstrated undifferentiated epithelial tumors. Occasional xenografts demonstrated intracellular mucin in a few cells, or made feeble attempts at glandular formation. Xenografts of the NCI-H716 cell line had additional unusual features; s.c. tumors frequently invaded and destroyed surrounding muscle tissue, a feature not noted with other colorectal xenografts. In addition, when injected intraperitoneally, cell line NCI-H716 formed solid tumor masses with invasion and destruction of the pancreas and other organs.

The other three gastric lines, SNU-1, SNU-5, and SNU-16, displayed both adherent and float-

TABLE I. Characteristics of Colorectal Cancer Cell Lines

	NCI-H548	NCI-H630	NCI-H508	SNU-C1	SNU-C2A	SNU-C4	SNU-C5	NCI-H716	NCI-H498
ATCC number	CCL 249	—	CCL 253	—	CCL 250.1	—	—	CCL 251	CCL 254
Date of initiation	1/83	9/83	10/82	3/84	9/84	12/84	11/84	4/84	8/76
Age	52	60	55	71	43	35	77	33	56
Sex[a]	M	M	M	M	F	M	M	M	M
Race[b]	W	W	W	O	O	O	O	W	W
Blood type	A+	A+	A+	O+	A+	AB+	AB+	O+	A+
Prior therapy	None	FAM & R[c]	5-FU	None	None	None	None	5-FU	None
Primary tumor site	Sigmoid	Rectum	Cecum	Descending	Cecum	Transverse[d]	Cecum	Cecum	Ileocecum
Cultured tumor site	Primary	Liver	Abd. W.	Peritoneum xenograft	Primary xenograft	Primary xenograft	Primary	Ascites	Peritoneum
Differentiation	Well	Well	Moderate	Moderate	Poorly	Poorly	Poorly	Poorly	Mucinous
Original tumor differentiation	Well, carcinoid	Moderate	Well	Moderate	Moderate	Moderate	Poor	Poor	Moderate/well: mucinous
Substrate adherence									
R10	+	+	+	±	+	+	+	−	+
ACL-4	−	−	−	±	−	+	+	−	−
Culture appearance[e]	G, D	G	G	G/U	U	U	U	U	G, D, ECM
Doubling time (h)	73	46	53	45	82	34	67	67	94
Plating efficiency (%)[f]	0	3.5	5.6	F	0.5	6.5	3.3	F	0.2
Cloning efficiency (colonies/10^5 cells)	0	800	8	1,300	5	310	380	2,500	5
Tumorigenicity	+	+	+	+	+	+	+	+	+
CEA cell extract	(−)	(+)	(+)	(+)	(+)	(+)	(±)	(−)	(+)
supernatant	(−)	(−)	(+)	(+)	(−)	(+)	(−)	(−)	(+)
CA19-9 cell extract	(−)	(−)	(+)	(+)	(−)	(+)	(+)	(−)	(+)
supernatant	(−)	(−)	(+)	(+)	(−)	(+)	(+)	(−)	(+)
TAG-72 cell extract	(−)	(−)	(+)	(+)	(+)	(+)	(−)	(−)	(+)
supernatant	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
DDCh cell line[g]	52	103	247	133	5	10	6	64	99
DCG[h]	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)
CGA RNA expression[i]	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)
Chromosome no.									
Modal	59	65	102	78	46	47	48	61	51
Range	55–63	63–69	71–131	76–83	44–48	46–47	46–53	55–64	47–54
Polyploid (%)	3	7	4	4	16	2	9	1	5
% of metaphases with DM chromosome	62	100	94	100	4	0	4	100	0
c-myc	+	+	+	+	+	+	+	+	+
N-myc	+	0	0	0	0	0	0	0	0
L-myc	0	0	0	0	0	0	0	0	0
myb	+	+	+	++	0	+	tr	tr	+
sis	0	0	0	0	0	0	0	0	0
IGF-II	+	0	0	0	0	0	0	0	0
GRP	0	0	0	0	0	0	0	0	0

[a]M, male; F, female. [b]W, white; O, oriental. [c]FAM, 5-FU + adriamycin + mitomycin-C; R, radiation. [d]Transverse, transverse colon. [e]G, gland like structure; D, dome formation; ECM, extracellular mucin secretion; U, undifferentiated. [f]F, not done due to lack of substrate adherence. [g]DDC, L-dopa decarboxylase-activity. [h]DCG, dense core granule. [i]CGA, Chromogranin A.

TABLE II. Characteristics of Gastric Cancer Cell Lines*

	SNU-1	SNU-5	SNU-16	NCI-N87
ATCC number				
Date of initiation	4/84	6/87	7/87	8/76
Age	44	33	33	?
Sex[a]	M	F	F	M
Race[b]	O	O	O	W
Blood type	O+	O+	A+	?
Prior therapy[c]	None	FAM	None	None
Primary tumor site	Stomach	Stomach	Stomach	Stomach
Cultured tumor site	Stomach	Ascites	Ascites	Liver
Differentiation	Poor	Poor	Poor	Well
Original tumor differentiation	Poor	Poor	Poor	Well
Substrate adherence				
R10	–	–	–	–
ACL-4	NT	NT	NT	NT
Culture appearance[d]	U	U	U	U
Doubling time (h)	26	34	27	47
Plating efficiency (%)[e]	F	F	F	4.3
Cloning efficiency (colonies/10^5 cells)	190	2,700	1,000	—
Tumorigenicity	+	NT	NT	+
CEA cell extract	(+)	(+)	(+)	(+)
supernatant	(+)	(+)	(+)	(+)
CA19-9 cell extract	(+)	(+)	(+)	(+)
supernatant	(+)	(+)	(+)	(+)
TAG-72 cell extract	(+)	(+)	(+)	(+)
supernatant	(−)	(−)	(−)	(−)
DDC cell line[f]	NT	87	87	NT
DCG[g]	NT	NT	NT	NT
CGA RNA expression[h]	NT	NT	NT	NT
Chromosome no.				
Modal	47	89	92	43
Range		85–98	81–94	38–45
Polyploid (%)	5.2	9.6	1.0	16.0
% of metaphases with DM chromosome	28	16	12	64
c-myc	+	+	+	+
N-myc	0	0	0	0
L-myc	0	0	0	0
myb	0	0	0	0
sis	0	0	0	0
IGF-II	0	0	0	0
GRP	0	0	0	0

*NT, not tested.
[a]M, male; F, female.
[b]W, white; O, oriental.
[c]FAM, 5-FU + adriamycin + mitomycin-C.
[d]U, undifferentiated.
[e]F: not done due to lack of substrate adherence.
[f]DDC, L-dopa decarboxylase-activity.
[g]DCG, Dense core granule.
[h]CGA, Chromogranin A.

ing subpopulations (Table II), and the cells appeared more rounded. However, SNU-16 cell line formed small numbers of globlet cell in culture.

Mucinous Cell Line (NCI-H498)

In ACL-4 medium, cell line NCI-H498 grew as floating aggregates surrounded by a halo of varying diameter, easily visible by phase microscopy. The halos stained strongly positive with the stains alcian blue, mucicarmine, and periodic acid-Shiff, confirming its mucinous nature. Mucin stains indicated varying degrees of intracellular mucin production by other cell lines and their corresponding xenografts, especially the

well-differentiated lines. However, prominent extracellular mucin secretion was a feature only present in NCI-H498 cells. The aggregates would not attach to the substrate in R10 medium unless the mucinous coat was dispersed by trypsinization. Monolayer cells were large and often distended with mucin droplets. Gland and dome formation were occasionally present. Xenograft histology and ultrastructural morphology indicated that the cell line had characteristics of a well-differentiated adenocarcinoma, and confirmed the prominent mucin secretion. Ultrastructural examination demonstrated abundant intra- and extracellular mucin. It could be adapted to an adherent line of the extracellular mucin coat, which was removed by trypsinization (and if attachment factors were present in the medium). Inoculation of this line intraperitoneally into athymic nude mice resulted in a similar situation: glandular structures surrounded by a mucin coat. The appearances closely mimicked pseudomyxoma peritonei, a clinical condition characteristic of mucinous carcinoma, showing extensive peritoneal involvement without invasion of abdominal organs. The tumor cells formed floating cell aggregates with gland formation surrounded by mucinous coats, closely mimicking their cultural appearance in ACL-4 medium. Pleural metastases were frequently noted after intraperitoneal inoculation.

OTHER CHARACTERISTICS

Expression and Secretion of Antigens

All colorectal lines were tumorigenic when inoculated s.c. into athymic nude mice and at least two grew i.p. CEA, CA 19-9, and TAG-72 were expressed by 67% (6/9), 56% (5/9), and 44% (4/9), respectively. Lines expressing CEA and CA 19-9 actively secreted these antigens into the supernatant fluids, while TAG-72 antigen was not secreted. Our findings suggest that CEA and CA 19-9 may function as more sensitive serum markers than TAG-72. CEA, CA 19-9, and TAG-72 were also found in cell pellets of 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. Only one gastric carcinoma cell line (SNU-1) had a detectable concentration of TAG-72 antigen in supernatant fluid (900 ng/10^6 cells). As with colorectal cell lines, there was no correlation between degree of morphological expression by these gastric lines and antigen concentrations.

Neuroendocrine (NE) Cell Features

Many of the colorectal cell lines expressed varying concentrations of dopa decarboxylase (DDC), a key neuroendocrine (NE) cell marker [16]. DDC is essential for formation of biogenic amines, both serotonin and catecholamines. The incidence and concentration of DDC increased after in vitro growth. These data are in agreement with a large survey of human tumors that indicated that non-small cell lung cancers (12%) and colorectal tumors (51%) were the only none-NE tumor types to frequently express DDC. The range of DDC values in the colorectal cell lines was similar to that present in cell lines derived from small cell carcinoma of the lung [17], a typical NE tumor. Cell line NCI-H716 may constitute another possibly unique subtype, an undifferentiated line with cytoplasmic dense core granules, characteristic of endocrine secretion, present in every cell. While osmiophilic granules have been described in COLO 320 and COLO 321 [18] derived from the same tumor, the illustrations are of low power and cannot be critically evaluated. Because dense core granules, characteristic of NE cells, were not present in other DDC positive lines, NCI-H716 only express part of the NE program.

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

Receptor Bindings

Peptide binding studies were performed using 12 different gastrointestinal hormones or neurotransmitters in 8 out of 9 colorectal cancer cell lines. All of the cell lines exhibited binding for at least one of the radioligands studies. Five of the 8 cell lines exhibited a significant amount of binding of ^{125}I-VIP, and five cell lines exhibited a significant amount of binding of [^{3}H]NMS. The results of radiolabeled peptides saturably bound to our colorectal cancer cell lines are summarized in Table III. NCI-H716 exhibited binding

for 7 radioliglands, while NCI-H630 and SNU-C4 exhibited binding for only one radioligand each. Analysis of ^{125}I-VIP binding on cell line SNU-C4 revealed a receptor having 2 classes of binding sites: one of high affinity (Kd 3.6 nM) and one of low affinity (Kd 1.7 μM) which represented the majority of the 5.5 × 10^6 binding sites/cell. Analysis of [^{3}H]NMS binding on cell line NCI-H508 revealed a Kd of 0.2 nM for N-methylscopolamine with a binding capacity of 2,500 sites/cell. With the agonist carbamylcholine, the receptor exhibited 2 classes of binding sites: one of high affinity (Kd 55 μM) representing 75% of the binding sites and one of low affinity (Kd 0.3 mM) representing 25% of the binding sites. Analysis of ^{125}I-[Tyr^4]-bombesin binding on cell line NCI-H716 revealed a high affinity receptor (Kd 2.1 μM) with a binding capacity of 3,300 sites/cell. Evaluation of biological activity mediated by the muscarinic cholinergic and bombesin receptors revealed an increase of intracellular calcium and of inositol triphosphate by specific receptor agonists. These results demonstrate that bombesin receptors and muscarinic cholinergic receptors on the human colon cancer cell lines could alter intracellular mediators in the tumor cells.

In peptide binding test of gastric carcinoma lines, saturable binding of ^{125}I-VIP was seen in SNU-1, SNU-5, and SNU-16 but was minimally detectable in NCI-N87. With 50 pM ^{125}I-VIP, the saturable binding as a percentage 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 [^{3}H]-NMS was seen in SNU-5 and NCI-N87 but not in SNU-1 or SNU-16 (Table IV). With 0.6 nM [^{3}H]-NMS, the saturable binding as a percentage 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 IV). To further examine the interaction of ^{125}I-VIP with receptors on SNU-1, the ability of VIP and secretin to inhibit binding of ^{125}I-VIP was studied. VIP caused detectable inhibition at 0.3 nM, half-maximal inhibition at 6.3 nM, and complete inhibition at 0.3 μM. Secretin, although structurally related to VIP, failed to inhibit binding of ^{125}I-VIP at concentrations of 0.1 μM, demonstrating it was as least 3,000

TABLE IV. Binding of Radiolabeled Peptides to Human Gastric Carcinoma Cell Lines*

Radiolabeled peptide added	Radiolabeled peptide saturably bound (fmol/g DNA)			
	NCI-N87	SNU-1	SNU-5	SNU-16
^{125}I-VIP	9.4	648	29	61
^{125}I-BH-SP	ND	ND	ND	ND
^{125}I-Gastrin-17-1	ND	ND	ND	ND
^{125}I-Tyr^4-bombesin	ND	ND	ND	ND
^{125}I-Neuromedin B	ND	ND	ND	ND
^{125}I-CGRP	ND	ND	ND	ND
^{125}I-OH-BZP	ND	ND	ND	ND
[^{3}H]NMS	85	ND	69	ND

*BH-SP, Bolton-Hunter-labeled substance; CGRP, calcitonin gene-related product; OH-BZP, hydroxybenzopindolol; NMS, N-methylscopoiamine; ND, not detected.

TABLE III. Binding of Radiolabeled Peptides to Human Colorectal Cancer Cell Lines*

Radiolabeled peptide added	Radiolabeled peptide saturably bound (fmol/μg DNA)							
	NCI-H548	NCI-H630	NCI-H508	SNU-C1	SNU-C4	SNU-C5	NCI-H716	NCI-H498
^{125}I-VIP	ND	76	111	38	99	61	ND	ND
^{125}I-BH-SP	ND	ND	ND	ND	ND	ND	22	ND
^{125}I-BH CCK 8	70	ND	ND	ND	ND	ND	69	16
^{125}I-Gastrin 17 1	43	ND	ND	ND	ND	ND	ND	ND
^{125}I-Tyr^4-Bombesin	ND	ND	ND	ND	ND	17	294	67
^{125}I-Neuromedin B	ND	ND	ND	ND	ND	ND	3.5	ND
^{125}I-CGRP	ND	ND	ND	ND	ND	4.4	44	ND
^{125}I-OH BZP	ND	ND	ND	ND	ND	ND	ND	ND
^{125}I-Secretin		ND	ND	ND	ND	ND	ND	ND
^{125}I-Tyr11-Somatostatin-14	ND	ND	ND	ND	ND	ND	246	ND
[^{3}H]NMS	314	ND	1,561	1,270	ND	ND	3,443	399
[15,16 ^{3}H]Etorphine	ND	ND	ND	ND	ND	ND	ND	ND

*ND, not detected. BH-SP, Bolton-Hunter-labeled substance; CGRP, calcitonin gene-related product; OH-BZP, hydroxybenzopindolol.

times less potent than VIP and that ^{125}I-VIP was in fact binding to the VIP receptor and not to secretin receptor. When the data was analyzed using a nonlinear least squares curve-fitting program, LIGAND [19], the inhibition of binding of ^{125}I-VIP by VIP was best fit with a one-receptor model having a high affinity for VIP (Kd = 2.5 nM).

Although it is unclear as yet, the fact that a greater number of receptors for hormones and neurotransmitters were exhibited on human colorectal cancer cell lines compared to gastric cancer cell lines, could raise the possibility that gastrointestinal hormones may have a greater effect on colon cancer cell growth [20].

Cytogenetic Studies

Each gastric cancer cell line had unique modal karyotypic characteristics, indicating its independent origin. 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 ploidies, 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 range (i.e., excluding the higher ploidies) was obvious only in SNU-16, two distinct sublines (in each cell line) could be identified by chromosome hybrid analysis. Each subline had a unique and consistent subset of chromosome that distinguished it. Generally, these differences totaled less than 10% of the chromosome complements.

Cytogenic studies performed on colorectal cancer cell lines showed that one of the lines examined was pseudodiploid, while others were aneuploid with modal chromosome numbers varying from 47 to 102.

DMs were found in 12–64% of the cells of all gastric lines and in 7 of 9 colorectal lines (Tables I, II). But only one or two DMs/cell were seen in all gastric lines except SNU-16, and only a portion of cells in all gastric lines possessed this aberration. In SNU-16, 4 to over 20 DMs were detected in some cells in both major and minor sublines. In one colorectal cell line some of the DMs were larger than usual, as described by Bullerdiek et al. [21], and are termed chromatic blocks.

HSRs were present in the minor subline of SUN-16 and 3 colorectal lines, all from previously treated patients. SNU-16 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. The 11p HSR was the most common of the HSR chromosomes, whereas 9q HSR occurred only once.

DMs and HSRs are characteristics of cells having amplified gene sequences, especially proto-oncogenes and genes associated with drug resistance. Previous reports have indicated that the finding of DMs and HSRs in colon carcinoma lines is infrequent [22]. However, COLO 320, a colon line reputed to have neuroendocrine properties, had many DMs which were later replaced by an HSR [18].

Gene Amplification and Expression

The myc gene family are among the proto-oncogenes frequently associated with cytogenetic evidence of gene amplification.

In one gastric cell line, SNU-16, an intense signal corresponding to the 12.7-kb EcoRI c-myc fragment was detected. The signal from this line was estimated to be 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 erb-B RNA that were comparable to the levels detected in a panel of colorectal carcinoma cell lines. Of interest, the level of c-myc RNA expression by the SNU-16 cell line was comparable to those expressed by the other three cell lines. No expression of the following genes by any of the gastric carcinoma cell lines was detected: N-myc, L-myc, C-sis, IGF-II, and gastrin releasing peptide.

Among the colorectal lines, c-myc amplification was present in COLO 320 [23]. While all of the colorectal lines expressed c-myc mRNA, only one line, NCI-H716, had amplified gene sequences. We also tested our colorectal cell lines for proto-oncogene and growth factor mRNA expression. Analysis of the proto-oncogene expression revealed detectable levels of c-myc, EGF-R, and K-ras message in all colorectal lines. The degree of c-myc expression was relatively uniform between the cell lines regardless of the source (primary vs. metastasis), the histologic grade, or location of the primary tumor. EGF-R expression, however, varied in intensity. K-ras mRNA expression was uniform between colorectal cell lines with the exception of NCI-H630, which demonstrated a strikingly increased level of K-ras expression; Southern blotting analysis confirmed the presence of about 100-fold ampli-

fication of the K-ras gene. This line was also remarkable for the presence of HSRs. This is the only cell line which had originated from a site that had been previously irradiated and then subsequently progressed. Carmichael et al. [24] recently analyzed the radiosensitivity of four of these cell lines including NCI-H630, which turned out to be the most radio-resistant of the four. Thus, the presence of increased K-ras mRNA expression may predict for increased resistance of the tumor to radiotherapy. Detectable levels of the 4.6 kb Her-2 message could be seen in 7 of the 9 colorectal cell lines whereas detectable c-sis message (4.2 kb) was found in 2 of the 9 cell lines, SNU-C2 and SNU-C5, both of which are poorly differentiated cell lines and were derived from xenografts. Expression of c-myb was found in 8 of the 9 colorectal cell lines, whereas N-myc expression was limited to one of the cell lines (NCI-H548). No evidence of L-myc expression was detected in any of the 9 colorectal cell lines. Significant variability between the colorectal cell lines was observed with regard to growth factor mRNA expression. IGF-II transcripts were clearly detected in NCI-H548. TGF-β mRNA was found in 7 of 8 colorectal cell lines tested with marked variability in the degree of expression among those eight cell lines. The highest level of expression was seen in NCI-H716, whereas only trace levels were seen in SNU-C2 and NCI-H630. No gastrin-releasing peptide (GRP) mRNA could be detected.

On the basis of their relative signal intensity and frequency of expression, proto-oncogenes and growth factor genes of colorectal carcinoma cell lines can be grouped into four categories: (1) genes whose level of expression is relatively uniform in all the colorectal cell lines (c-myc); (2) genes which are detectable in most, if not all the colorectal cell lines but with variation in signal intensity (K-ras, Her-2, c-myb, EGF-R, and TGF-β); (3) genes whose level of expression was detected in less than 50% of the colorectal cell lines (N-myc, c-sis, and IGF-II); and (4) genes whose expression was not detectable in any of the colorectal cell lines (L-myc and GRP). Although heterogeneity of proto-oncogene and in particular growth factor gene expression was detected among the colorectal cell lines, no statistically significant associations could be made between the pattern of gene expression of a cell line and the known biological and clinical parameters. However, K-ras was amplified in a cell line derived from a radioresistant tumor (NCI-H630), while detectable N-myc expression was found in a cell line derived from a tumor with neuroendocrine features (NCI-H548).

p53 transcripts of the expected 2.8 kb size were readily detected in two (SNU-16 and NCI-N87) of the four gastric cell lines by Northern blot analysis. No transcript was detected in cell line SNU-1 established from a primary tumor, and cell line SNU-5 established from a metastasis. Sequence analysis of p53 mRNA by cDNA/PCR demonstrated abnormalities in all four gastric cell lines. The finding of a 24 bp deletion at the 5′ end of exon 8 in the cDNA of the SNU-5 cell line suggested an abnormal mRNA splicing event. A point mutation in the splice acceptor site at the 3′ end of intron 7 was found. This intronic point mutation converts a splice acceptor sequence, 5′-GAGTAG-3′, into the sequence 5′-GAGTCG-3′. The point mutation appears to abolish normal splicing at this site and results in complex abnormal splicing which utilizes a cryptic splice acceptor site (5′-GAACAG-3′), located near the 5′ end of exon 8, resulting in a 24 bp deletion in the mRNA of this cell line.

Chemosensitivity

In vitro chemosensitivity of colorectal cancer cell lines was determined using the semiautomated tetrazolium-based MTT colorimetric assay. It has been reported that the MTT assay yields results very similar to the clonogenic tumor stem cell assay [25]. The drugs used were: 5-FU, doxorubicin, cisplatin, melphalan, etoposide, mitomycin-C, and BCNU. IC_{50} value was defined as the concentration of drug which produced 50% reduction of absorbance at 540 nm. From assay results, we predict 5-FU to be the sole active agent of the seven tested. The range of drug concentrations which produced 50% of cell growth was greatest with 5-FU (388-fold vs. 5- to 30-fold with the other six agents), and the area under the curve (AUC) which produced 50% growth inhibition was within a clinically achievable range only for 5-FU. Since the assay AUC of 5-FU at 50% inhibition was in a clinically achievable range for only two of the cell lines (SNU-C1 and SNU-C4), we performed a multivariate analysis to explore parameters which predict 5-FU sensitivity. As a result of a multiple regression analysis, concentration of 5-FU is inversely related to % cloning efficiency in media and TAG-72 antigen expression.

We also tested the effect of 5-FU and 5-fluoro-2′-deoxyuridine (FdUrd) with and without leucovorin (LV) on our panel of nine colorectal cancer cell lines using MTT assay [26]. The

effect of leucovorin on 5-FU and FdUrd was quantatively similar. A clinically achievable level of LV (20 μM) increased the cytotoxicity in all three replicate experiments in 8 of the 9 cell lines ($P < 0.05$, binomial test). LV alone at a concentration of 20 μM had no effect on cell survival. In this experiment, 3 cell lines (SNU-C1, SNU-C4, and NCI-H508) had 50% inhibition of growth occurring at a clinically achievable AUC of 5-FU alone. With the addition of LV, one additional cell line (NCI-H498) showed 50% growth inhibition at a clinically achievable level of 5-FU. These results clearly demonstrates that LV increases the cytotoxicity of these agents for the colorectal cancer cell lines.

In vitro chemosensitivity of 4 gastric cancer cell lines were also performed using the MTT assay. In comparison with the colorectal cancer cell lines, the three poorly differentiated gastric cell lines (SNU-1, SNU-5, and SNU-16) were relatively sensitive to doxorubicin, mitomycin, and cisplatin, and their range of 50% inhibitory concentration (IC_{50}) values was relatively narrow, whereas the well-differentiated gastric cancer cell line was relatively resistant. The responses of the gastric cancer cell lines to 5-FU and floxiuridine were similar to those of the colorectal cancer cell lines, and the IC_{50} value had a wide range. However, the well-differentiated gastric cancer cell line also was more resistant to these drugs than the poorly differentiated lines. When the assay AUC values were calculated from the IC_{50} values, three of the gastric cancer cell lines had assay AUC within a clinically achievable AUC for doxorubicin and cisplatin. Only one gastric cancer cell line met this criterion for 5-FU and floxuridine. Addition of leucovorin at a clinically achievable concentration enhanced the cytotoxic effects of both fluorouracil and floxuridine in colorectal cancer cell lines, but it enhanced the effects of only floxuridine in gastric cancer cell lines.

With the use of a slot blot assay, relatively low levels of MDR1 mRNA were present in all four gastric cancer cell lines, while intermediate or high levels were present in most of the colorectal cancer cell lines. These findings suggest that some, but not all, of our observations may be related to the relatively low levels of MDR1 RNA present in gastric cancer and cell lines.

CONCLUSIONS

From a clinical view point, colorectal and gastric carcinomas are very different forms of malignancy. However, the multiple properties of gastric carcinoma cell lines are remarkably similar to those found in a panel of colorectal carcinoma cell lines. 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, there are certain differences in the biology of these two tumor types, which may reflect the clinical differences. 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. In addition, colorectal carcinoma cell lines express receptors for peptide hormones more frequently than gastric carcinoma cell lines.

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