Clinical Significance of Gastrin Receptors in Human Colon Cancers

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ABSTRACT

We have measured gastrin receptors (GR) in surgical specimens from 67 patients with primary colon cancers in order to determine the clinical significance of GR in colon cancer. GR analysis was performed on these specimens, and 22 cancers (32.8%) had no detectable GR. Thirty-eight cancers (56.7%) had high-affinity (Kd < 1.0 nm) levels of GR. Seven cancers (10.4%) had only low-affinity GR (Kd > 1.0 nm). Twenty patients (29.9%) had cancers with GR > 10 fmol/mg protein. Mean GR content was significantly greater (11.8 ± 2.9 fmol/mg protein) in Dukes' Stage A and B cancers when compared to Stage C and D cancers (6.2 ± 1.6 fmol/mg protein). A significantly greater percentage (52.4%) of patients in the early stages (A and B) had tumors with >10 fmol/mg protein compared to patients with more advanced (C and D) cancers (19.6%). GR content did not correlate with histological differentiation, patient age, or preoperative carcinoembryonic antigen levels. No difference in the GR content was noted between left and right colon cancers or in patients of different sex or race. GR content of normal colon mucosa correlated with the GR content of colon cancers from the same surgical specimen, suggesting that these tumors maintain their normal complement of GR. In the early period of follow-up, 12 of 43 (28%) Stage C and D patients with GR < 10 fmol/mg protein have died, whereas all 8 Stage C and D patients with GR > 10 fmol/mg protein are alive. GR content of colon cancers may have prognostic significance and may identify a group of patients with colon cancer that may benefit from hormonal therapy with anti gastrin drugs.

INTRODUCTION

Colon cancer is second only to lung cancer as a cause of cancer death in the United States; approximately 145,000 new cases occur and 60,000 Americans die of this disease annually (1). Treatment of colon cancer relies heavily upon surgical resection. No widely effective adjuvant therapy is presently available. Prognosis for survival is determined primarily by stage of disease at the time of diagnosis (2, 3). Since the majority of patients with colon cancers have serosal penetration and nodal involvement at the time of operation, effective methods of systemic therapy are needed.

The relationships among hormones, growth factors, their receptors, and malignant cells have been recently reviewed (4-6). Gastrin is a gastrointestinal hormone produced by G-cells (mainly in antral mucosa) that stimulates gastric acid secretion and the growth of portions of the normal gastrointestinal tract in rats (7) and mice (8). Patients with gastrinomas have hyperplasia of parietal cells of the gastric mucosa (9). Gastrin and other peptide hormones act via specific membrane receptors. Gastrin receptors are present on fundic, duodenal, and colonic mucosal membranes of rats (10, 11) and on parietal cells from dogs (12). We have previously reported that normal human gastric and colonic mucosa and adenocarcinomas of the human stomach and colon contain specific receptors for gastrin (11, 12). The functional significance of these receptors on these human cancers is not yet clear.

Winsett and coworkers (8) found that treatment with pentagastrin, an analogue of gastrin, enhanced growth of a mouse colon carcinoma and decreased the survival of treated mice. Beauchamp and coworkers (14) reported that proglumide, a gastrin and cholecystokinin receptor antagonist, inhibited colon cancer growth and prolonged survival in tumor-bearing mice. Chronic endogenous hypergastrinemia, induced by antral exclusion in rats, significantly increased carcinogen-induced growth of colon cancer (15). Human colon cancers growing in athymic nude mice (16) and in tissue culture (17-19) have been stimulated by gastrin.

The purpose of the present study was to determine the clinical significance of gastrin receptors on human colon cancers. We used the technique for gastrin receptor analysis which we developed (11) on freshly resected surgical specimens from patients with primary colon cancers. We examined the relationships between the gastrin receptor content and patient age, race, sex, site of colon carcinoma, preoperative CEA level, histological differentiation, stage of disease, early recurrence, and survival.

MATERIALS AND METHODS

Specimens of primary colon carcinomas were obtained at the time of surgical resection from 67 patients treated at the University of Texas Medical Branch Hospitals. The age, sex, race, and preoperative level of CEA, as well as site of colon carcinoma, were recorded. The colon was divided into left and right sides according to embryonic development: the right side (mid-gut) included the cecum, ascending colon, and transverse colon; the left colon (hind-gut) included the splenic flexure, descending colon, sigmoid, and rectum.

All specimens were examined by the Surgical Pathology Service. Immediately upon removal of the surgical specimens, portions of viable cancer and colon mucosa were excised for measurement of gastrin receptors. Frozen sections of the cancer adjacent to the excised portion were examined for confirmation of viable cancer. Normal mucosa was obtained from the margin of the resected colon; the absence of cancer at the margin was confirmed histologically.

The histological diagnosis of colon carcinoma, the degree of differentiation of the tumor, and the Astler-Coller modification of Dukes' classification of the stage of disease (20) were determined by a surgical pathologist who had no knowledge of the results of gastrin receptor analysis. Mucoid adenocarcinomas were classified separately because these tumors have a particularly bad prognosis, even if the tumor is well differentiated and no lymph node metastases are present (21).

Specimens for Gastrin Receptor Analysis. The content of gastrin receptors of the colon cancers and of normal colon mucosa from the surgical specimen was determined by methods we have previously described (11). Briefly, portions of the cancer and normal mucosa were excised and washed in ice-cold Buffer A [10 mm Tris: 137 mm NaCl: 5 mm KCl: 2 mm CaCl2: 2.5 mm MgCl2: 0.25 mm sucrose (pH 7.4)], containing 0.1% BSA (Fraction V; Sigma). Specimens were placed in storage vials, snap frozen in liquid nitrogen, and stored at -70°C in an ultradry-freeze (Revco). Frozen tissues were weighed and pulverized with a Thermovac autopulverizer cooled in liquid nitrogen. The resulting powder was homogenized in 5 volumes of Buffer A plus BSA, using a precooled Polytron homogenizer (PG-10ST; Brinkman Industries, 

The abbreviations used are: CEA, carcinoembryonic antigen; BSA, bovine serum albumin; GBS, gastrin binding sites.
CLINICAL SIGNIFICANCE OF GASTRIN RECEPTORS

Westbury, CA). The homogenate was filtered through a double layer of cheese cloth, presoaked in ice-cold Buffer A, and subjected to 200 × g 10-minute centrifugation to remove any cell debris. For the preparation of total crude membrane fractions, the resulting supernatant was subjected to 30,000 × g 45-min centrifugation in a fixed-angle SM-24 rotor. The resulting pellets were then washed once again with Buffer A plus BSA and repelleted at 30,000 × g for 45 min. The pellet obtained was resuspended in Buffer B (25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid:2.5 mM MgCl· 5 mM KCl; 137 mM NaCl; 0.7 mM KH2PO4; 10 mM glucose (pH 7.4)) plus BSA, homogenized manually with a glass Teflon homogenizer, and processed for measurement of protein (22) and gastrin receptors.

Gastrin Receptor Assay. Synthetic human gastrin-17-1 (Bachem, Torrance, CA) was iodinated as we have previously described (11). The biological activity of iodinated molecules is similar to that of an equimolar concentration of noniodinated gastrin. Aliquots of the membrane suspension in Buffer B plus BSA containing approximately 200 μg of protein were used as substrate in each tube. A multipoint saturation analysis (7 to 10 points per assay), using increasing concentrations of radiolabeled gastrin (0.01 to 0.5 nM) with (nonspecific binding) or without (total binding) 1000-fold excess of radioinert gastrin, was carried out, and the binding was analyzed by a Scatchard plot of the specific binding data. The binding assay was performed at 30°C in a water bath for 30 min, after which the tubes were chilled on ice, and 100 μl of ice-cold Buffer B plus BSA were added. The peptide hormone bound to the substrate was separated from the excess unbound hormone by filtration over cellulose acetate filters (Celorate; Millipore) using a multifiltration unit (1225 sampling manifold; Millipore). The membrane filters containing the substrates were placed in a glass tube and counted in a gamma counter (Beckman, Model 5550, with 78% efficiency for 125I counting).

Data Analysis. The relationship between the age of the patient and the preoperative CEA levels compared to the gastrin receptor content was examined by means of linear regression analysis. The mean gastrin receptor content was determined in groups of patients with different race, sex, degree of histological differentiation of cancers (well- and moderately differentiated cancers versus poorly differentiated and mucinous cancers), and stage of disease (Stage A and B versus Stage C and D). Differences between groups were analyzed by the Kruskal-Wallis test and the Mann-Whitney test, and a P < 0.05 was considered significant. Histological differentiation, stage of disease, and site of colorectal cancer were also compared between patients who had cancers positive for >10 fmol/mg protein of specific gastrin receptors and patients who had cancers with <10 fmol/mg protein of specific gastrin receptors by means of Fisher’s exact test. The content of gastrin receptor of normal colon mucosa was compared to the content in colon cancers by linear regression analysis. To determine whether gastrin receptors may be of prognostic significance, early follow-up data have been obtained, and all cancer recurrences and deaths have been recorded.

RESULTS

In normal mucosa and cancers, that were found to be positive for specific gastrin binding sites, a single class of high-affinity gastrin binding sites (Kd = 0.1 to 0.3 nM) was observed on membrane preparations (Fig. 1). As may be seen from Fig. 1A, tumors with >10 fmol/mg protein of gastrin binding sites demonstrated specific binding as the major component of total binding, especially at subsaturating concentrations of the ligand, while tumors with <10 fmol/mg protein (Fig. 1B) invariably demonstrated nonspecific binding as the major component, even at subsaturating concentrations of the ligand. In many tissues, a second class of nonsaturable specific gastrin binding sites was also observed (Fig. 1), which we have as yet not characterized due to the paucity of available tissue. The number of type I, high-affinity, saturable gastrin binding sites on human tumor membranes was defined and correlated with various clinical parameters.

Of the 67 patients in the study, 35 were men and 32 were women. Forty-five had cancers arising from the left side of the colon, and 22 had cancers arising from the right side of the colon. Only one patient had a Stage A cancer, 20 were Stage B, 27 were Stage C, and 19 were Stage D.

Twenty-two patients (32.8%) had cancers with no detectable (<1.0 fmol/mg protein) high-affinity (Kd = <1.0 nM) gastrin binding sites, which we have called gastrin receptors. Thirty-eight patients (56.7%) had cancers with specific high-affinity gastrin binding sites (gastrin receptors) that ranged from 1.5 to 50 fmol/mg protein (Table 1). An additional 7 patients also had no detectable high-affinity gastrin receptors (10.4%), but had cancers with a significant number of low-affinity...
CLINICAL SIGNIFICANCE OF GASTRIN RECEPTORS

Table 1

<table>
<thead>
<tr>
<th></th>
<th>GR $^a$ ($K_a &gt; 1.0$ nM)</th>
<th>GBS ($K_a &gt; 1.0$ nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>Negative 22 (32.8)$^b$</td>
<td>Positive 38 (56.7)</td>
</tr>
<tr>
<td></td>
<td>Positive 7 (10.4)</td>
<td></td>
</tr>
<tr>
<td>Normal mucosa</td>
<td>Negative 22 (37.3)</td>
<td>Positive 28 (47.5)</td>
</tr>
<tr>
<td></td>
<td>Positive 9 (15.2)</td>
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$^a$ GR, gastrin receptors (negative = <1 fmol/mg protein; positive = >1 fmol/mg protein).

Of the 38 patients with specific, high-affinity gastrin receptors, 20 (30% of all patients) had >10 fmol of specific gastrin receptors/mg protein. All other patients had either <10 fmol of high-affinity gastrin receptors/mg protein or only low-affinity GBS. The gastrin receptor content of colon cancers did not correlate with patient age or preoperative CEA level, and the mean gastrin receptor content of tumors between males and females and among different races was not significantly different.

The mean gastrin receptor content in cancers from patients whose cancers were Stage A and B (11.8 ± 2.9 fmol/mg protein) was significantly greater than the content of those cancers which were Stage C and D (6.2 ± 1.5) (Fig. 2). A significantly greater proportion of patients with Stage A or B cancers (11 of 21 or 52.4%) had concentrations of >10 fmol gastrin receptor/mg protein compared with patients with Stage C and D cancers (9 of 46 or 19.6%) (Fig. 3).

The mean content of gastrin receptors of patients with well- and moderately differentiated tumors (8.6 ± 1.7 fmol/mg protein) was not significantly different from the gastrin receptor content of patients with poorly differentiated and mucinous adenocarcinomas (6.0 ± 2.8 fmol/mg protein) (Fig. 4). Of the 51 well- and moderately differentiated tumors, 17 (33%) had no detectable gastrin receptors, 6 (12%) had only low-affinity gastrin binding sites, 10 (20%) had detectable levels of gastrin receptors (<10 fmol/mg protein), and 18 (35%) were highly positive for gastrin receptors (>10 fmol/mg protein). All but two poorly differentiated and mucinous adenocarcinomas were either negative for gastrin receptors or had low levels (<10 fmol/mg protein) of gastrin receptors.

A greater percentage of patients (33.5%) with left-sided colon cancers had >10 fmol/mg protein of gastrin receptors, while only 22.7% of right-sided lesions had >10 fmol/mg protein of gastrin receptors. These differences are due to differences in stage of disease and not site of the cancer (Fig. 5). With right colon cancers, 42.9% of Stage A and B cancers had >10 fmol/mg protein of gastrin receptors, while only 13.3% of Stage C and D cancers were highly positive for gastrin receptors. With left colon cancers, 57.1% of Stage A and B tumors were highly positive for gastrin receptors (>10 fmol/mg protein) compared to 22.6% of tumors with Stage C and D.

The content of gastrin receptors of normal colon mucosa was determined in 59 of the 67 patients studied. Normal colon mucosa from these patients had a range of specific binding for
gastrin receptors which was similar to the colon cancers (Table 1). Twenty-two patients (37.3%) had no detectable gastrin receptors, while 28 patients (47.5%) had high-affinity receptors. Nine patients (15.2%) had only low-affinity binding sites. Most patients with normal mucosa with no detectable gastrin receptors also had colon cancers with no detectable gastrin receptors. Gastrin receptor content of the normal colon mucosa correlated significantly \( (P < 0.001, r = 0.6) \) with the gastrin receptor content of the colon cancers (Fig. 6). These data suggest that gastrin receptor content of normal colon mucosa may determine the gastrin receptor content in cancers.

There were 4 deaths within 30 days of operation (1 patient with a Dukes' B cancer, 2 with Dukes' C, and 1 with Dukes' D). These deaths were sudden deaths unrelated to cancer. Results of early follow-up of the remaining 63 patients are summarized in Table 2. The period of follow-up ranges from 40 to 972 days with a mean period of 292 days. All 21 patients with Stage A and B tumors are free of disease. Five of 19 (26.3%) Dukes' C patients with cancers negative or poorly positive (<10 fmol/mg protein) for gastrin receptors have developed recurrences, and 4 have died; none of the 6 Stage C patients with cancers highly positive for gastrin receptors has developed recurrence. Of the 16 Stage D patients with cancers negative or poorly positive for gastrin receptors, 8 (50%) have died of recurrent cancer; both of the Stage D patients with tumors highly positive for gastrin receptors are alive.

**DISCUSSION**

The palliative treatment of metastatic breast cancer by oophorectomy was reported initially by Beatson (23) in 1896. Unfortunately only 25 to 30% of human breast cancers are hormone dependent. Initial use of the estrogen receptor content of breast tumor biopsies as an indication of probable response of patients with advanced cancer to endocrine therapy was reported by Jensen and coworkers in 1971 (24); 10 of 13 patients (77%) with tumors highly positive for receptors responded to adrenalectomy, whereas only 1 of 29 (3%) with cancers with low levels of receptors responded. The presence of both estrogen and progesterone receptors increases the response rate of these tumors (25).

Huggins and Bergenstal (26) advanced the concept of hormonal manipulation for treatment of cancers arising from various hormone-responsive normal target tissues. They coined the terms hormone-dependent and hormone-independent for tumors which did and did not respond to hormone manipulation.

In the present study, we have shown that 56.7% of patients had specific gastrin receptors detected on their colon cancers. Thirty % of all patients had tumors with specific gastrin receptor levels that were greater than 10 fmol/mg protein detected in the cancers. Ten % of patients had cancers which were negative (<1.0 fmol/mg protein) for high-affinity gastrin receptors \( (K_d < 1.0 \text{ nm}) \) but had low-affinity \( (K_d > 1.0 \text{ nm}) \) gastrin binding sites, the significance of which remains to be determined. The gastrin receptor content in colon cancers correlated with the gastrin receptor content of the normal mucosa from the same patients, suggesting that these cancers retained the gastrin receptor complement of the normal mucosa from which these tumors arose. Most patients with no detectable gastrin receptors in normal mucosa also had no detectable receptors in the cancer. Similar levels were detectable in normal mucosa and colon cancers from the same patients (Fig. 6).

The significance of these observations is that cell proliferation in normal gastric and colonic mucosa may be controlled by gastrin, and the growth of colon cancers may also be affected by gastrin. We have found that the trophic effects of gastrin on MC-26 mouse colon cancers are mediated by regulation and maintenance of gastrin receptors on the cancer cells (27).

Estrogen and progesterone receptor analysis of primary breast cancers provides valuable prognostic information concerning the probability of recurrence of metastatic disease.
Women whose primary tumors are highly positive for receptors have a significantly longer disease-free interval in the absence of any systemic therapy (28) and a significantly longer survival (29).

Colon cancers that were without lymph node involvement or distant metastasis (Dukes' A and B) showed a significantly greater proportion of high gastrin receptor content and a significantly greater mean gastrin receptor content than did more advanced cancers. Early follow-up of these patients suggests that the gastrin receptor content of colon cancers may provide prognostic information. Long-term periods of follow-up will be required to determine whether gastrin receptors on colon cancers will provide prognostic information on the probability of recurrence and patient survival independent of stage of disease.

Surgical excision remains the only effective treatment for colorectal carcinoma. An effective treatment for advanced colon carcinoma has not been developed; there is no current widely effective systemic therapy (which can be used after resection) that can prolong survival of patients with colon cancer. Treatment with antigastrin compounds may be a promising method in selected patients.

REFERENCES

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