Original article

Prognostic significance of gastrin expression in patients undergoing R0 gastrectomy for adenocarcinoma

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Abstract

Background. Gastrointestinal (GI) hormones regulate several GI functions, including the proliferation and repair of normal mucosa, and hormone receptors may therefore be implicated in the growth, invasion, and metastasis of cancers of the GI tract. The aim of this study was to determine the cellular distribution of gastrin in intestinal-type gastric cancers, and to determine its relationship to outcomes after R0 gastrectomy. **Methods.** Eighty-six consecutive patients undergoing R0 gastrectomy for adenocarcinoma were studied. Normal gastric mucosa and tumor were stained for gastrin and their specific cellular distribution was determined.

Results. The duration of survival of patients whose tumors exhibited well-differentiated gastrin-positive tumor (GPT) cells ($n = 12$) was significantly poorer than that of patients whose tumors were GPT-negative (5-year survival, 30% vs 54%; $P = 0.037$). Patients with GPT-positive intestinal-type gastric cancer (5 of 47 patients) had the poorest survival of all (median, 14 months; 5-year survival, 0%; $P = 0.006$). In a multivariate analysis, only lymph node metastases (hazard ratio [HR], 2.13; 95% confidence interval [CI], 1.2 to 3.79; $P = 0.01$) and the presence of GPT cells (HR, 6.61; 95% CI, 1.74 to 25.09; $P = 0.01$) were independently and significantly associated with durations of survival in patients with intestinal-type gastric cancer.

Conclusion. The presence of GPT cells in patients with gastric adenocarcinoma is a significant and independent prognostic indicator.

Key words Gastrin · Gastric cancer

Introduction

The endocrine control of tumor growth was first identified by Beatson, a Scottish surgeon who reported the successful treatment of a patient with breast cancer by performing bilateral oophorectomies [1]. Subsequently, Charles Huggins, the Nobel Laureate for medicine, was instrumental in defining the endocrine control of breast and prostate cancers [2]. Other cancers have been shown to possess receptors for gastrointestinal (GI) hormones, which may then stimulate proliferation by endocrine, paracrine, or autocrine means [3]. Gastrointestinal hormones regulate several GI tract functions, including secretion, absorption, motility, and digestion. Certain GI hormones also play a role in the proliferation and repair of the normal GI mucosa, and it is becoming increasingly apparent that cancers of the stomach, colorectum, and pancreas possess GI hormone receptors, which are therefore implicated in the growth, invasion, and metastasis of cancers [4].

A century has now passed since the Cambridge physiologist John Sydney Edkins first identified a hormone responsible for gastric acid secretion in 1905, which he termed gastric secretin, or gastrin [5]. While the role of gastrin in this regard is now well defined, research interest has centered on the effect of the isoforms of gastrin in stimulating gastrin/cholecystokinin (CCK)-B receptors in certain types of gastric cancer. Recent studies have linked *Helicobacter pylori* infection and gastrin to risk factors for gastric cancer, and significantly higher levels of plasma and luminal gastrin have been reported in patients diagnosed with gastric cancer when compared with control patients. Henwood et al. [4] reported increased expression of gastrin and of the CCK-B/gastrin receptor in association with the progression of atrophic dysplasia to gastric adenocarcinoma. Given that *H. pylori* is a known risk factor for the development of gastric cancer, the association of high levels of gastrin with *H. pylori* infection is an important area of research.

Offprint requests to: W. G. Lewis

An original article presented in the Moynihan Prize session at the Associations of Surgeons of Great Britain and Ireland, Edinburgh, May 2006.

Received: February 5, 2007 / Accepted: May 19, 2007

Concerns have been raised that blind prescription of powerful acid-suppressing drugs, such as proton pump inhibitors (PPIs), may mask the symptoms of gastric cancer [6] and delay diagnosis [7]. Prolonged acid suppression secondary to PPI therapy leads to hypergastrinemia and hyperplasia of enterochromaffin-like cells in the gastric mucosa [8], although there is no evidence that this progresses to high-grade dysplasia or adversely influences outcomes [9]. There have been no studies investigating the relationship between PPI prescription, gastrin expression within patients' gastric tumors, and outcomes. The aim of this study was to determine the influence of gastrin on outcomes for patients with gastric cancer, and to investigate any possible relationship between this and hypergastrinemia induced by pharmacological acid suppression.

Patients and methods

Eighty-six consecutive patients undergoing potentially curative resection for gastric adenocarcinoma between October 1, 1995, and January 1, 2004, were studied. These patients were recruited from a consecutive series of 325 patients treated by the same multidisciplinary team in a single district general hospital. The demographic details of these patients are illustrated in Table 1. *H. pylori* status was assessed for each patient, using the *Campylobacter*-like organism (CLO) test (Kimberly-Clark, Kings Hill, Kent, UK). All patients were staged by means of computed tomography of the thorax and abdomen, supplemented with laparoscopy for those patients deemed potential surgical candidates. A modified D2 resection (preserving pancreas and spleen where possible) was the operation of choice [10, 11]. Patients were staged according to the unified TNM classification of gastric cancer [12, 13]. No patients received neoadjuvant chemotherapy.

Patients were followed up at 3-month intervals for the first year postoperatively and at 6-month intervals thereafter. Death certificates were obtained for all deceased patients ($n = 51$), confirming dates and causes of death. A total of 71 patients (83%) were followed up for at least 5 years or until death, and no patients were lost to follow up. Ethical approval was granted by Gwent Research Ethics Committee.

Laboratory and histopathological methods

Specimens of primary tumors and of nodal metastases taken from formalin-fixed specimens were obtained from the histopathology archives. All tumor sections were analyzed. Four-micrometer sections were cut from each specimen and mounted on glass slides. The slides were stored at 37°C and then baked at 65°C for 30 min

Table 1. Demographic details of the patients in relation to the presence of well-differentiated gastrin-positive tumor (GPT) cells

	GPT +ve	GPT -ve
<i>n</i>	12	74
Sex, M:F	9:3	49:25
Median age, years (range)	73 (55–79)	74 (27–86)
Blind PPI (%)	3 (25)	22 (30)
CLO test-positive (%)	4 (33)	19 (26)
Site of tumor (%)		
Cardia	4 (33)	21 (28)
Body	4 (33)	19 (26)
Antrum	4 (33)	32 (43)
Linitis plastica	0	2 (3)
Stage of disease (%)		
I	1 (8)	16 (22)
II	5 (42)	17 (23)
III	5 (42)	30 (41)
IV	1 (8)	11 (15)

CLO, *Campylobacter*-like organism

before use. Each section was deparaffinized with xylene and then rehydrated with graded alcohol and distilled water, before placement in a citrate buffer (pH 6) and pressure cooking for 5 min at full pressure. From the pressure cooker, the slides were transferred to an automated staining machine (I-6000; BioGenex, San Ramon, CA, USA) and incubated in 3% hydrogen peroxide for 10 min, then with DAKO protein block for 10 min. The slides were then incubated with 1/1000 rabbit anti-human gastrin antibodies, which react with mature amidated gastrin-17 and gastrin-34 (DakoCytomation; Denmark House, Ely, Cambridgeshire, UK), for 30 min, then with a supersensitive detection kit (labeled streptavidin biotin [LSAB]; DakoCytomation) for a further 30 min (15 min in the link reagent and 15 min in the label reagent). The slides were then treated with chromogen 3,3-diaminobenzidine tetrachloride (DAB; BioGenex) for 10 min, washed in running water for 5 min, counterstained with hematoxylin for 10 s, dehydrated with graded alcohols, cleared with xylene, and a cover slip applied. Slides were analyzed by a consultant histopathologist (I. T.), using a Leica DMRB microscope (Leica, Milton Keynes, Buckinghamshire, UK). The presence of gastrin within normal gastric mucosa and within the adenocarcinoma cells was noted for each patient. In addition, each tumor was scrutinized for the presence of well-differentiated cells containing gastrin (gastrin-positive tumor [GPT] cells; Fig. 1). Tumor cells were deemed to be well-differentiated if they contained a low ratio of nucleus to cytoplasm and few mitoses. The presence of a single GPT cell was sufficient for the patient to be classified as GPT-positive.

Slides containing GPT cells were incubated with anti-chromogranin antibodies as above and re-analyzed

for the presence of other, gastrin-negative, endocrine cells.

Statistical analysis

Statistical analysis appropriate for nonparametric data was used. Differences were deemed to be statistically significant when $P < 0.05$. The X^2 test for association was used to assess observed and expected frequencies. Grouped data were compared using the Mann-Whitney U (MWU) test for unpaired data. Correlations were tested using Spearman's rank correlation coefficient. Cumulative survival (using disease-specific mortality) was calculated by the life table method of Kaplan and Meier [14] and differences in survival times between groups of patients were analyzed by the log rank method. Cox's proportional hazards model was used to assess the prognostic value of individual variables. Data analysis was carried out using SPSS version 12 (SPSS, Chicago, IL, USA).

Results

Details of the patients and the surgery

The details of the patients are shown in Table 1. Forty-three patients (50%) underwent a subtotal gastrectomy and 43 patients (50%) underwent a total gastrectomy.

Prior therapy with acid-suppressing medication

A total of 25 patients (29%) were prescribed acid-suppressing medication prior to the diagnosis of gastric cancer. There was no significant difference between the cohorts of patients receiving prior PPI therapy or not with respect to stages of disease (MWU = 653.5; $P = 0.289$), the proportion of early gastric cancers (MWU = 656.0; $P = 0.159$), the proportion of patients with gastrin-staining tumor cells (MWU = 630.5; $P = 0.519$), or the presence of GPT cells (MWU = 741.5; $P = 0.739$).

Lauren classification

Forty-seven patients (55%) had intestinal-type gastric cancer and 39 patients (45%) had diffuse-type gastric cancer. Fifteen of the 47 patients (32%) with intestinal-type tumors had early gastric cancer, compared with 4 of the 39 patients (10%) with diffuse-type gastric cancer ($X^2 = 5.809$; df 1; $P = 0.016$). There was no association between Lauren classification and prior acid-suppression therapy with PPIs ($X^2 = 0.026$, df 1; $P = 0.872$), the presence of gastrin within normal cells ($X^2 = 0.291$; df 1; $P = 0.590$), the presence of gastrin in tumor cells ($X^2 = 0.258$; df 1; $P = 0.611$), or the presence of GPT cells ($X^2 = 0.949$; df 1; $P = 0.330$).

Gastrin expression in normal peritumor mucosal cells

Gastrin was identified in cells from the normal gastric mucosa of 62 patients (72%). There was no association between the presence of gastrin within normal cells and prior acid-suppression therapy with PPIs ($X^2 = 2.484$; df 1; $P = 0.115$), the presence of gastrin in tumor cells ($X^2 = 0.394$; df 1; $P = 0.530$), or the presence of GPT cells ($X^2 = 2.656$; df 1; $P = 0.103$).

Presence of gastrin within the primary tumor

Gastrin was identified within the tumor cells of 22 patients (26%). The presence of gastrin within tumor cells correlated strongly with the presence of GPT cells ($r = 0.480$; $P < 0.001$).

Presence of GPT cells within the primary tumor

Well-differentiated cells containing gastrin (GPT cells; Fig. 1) were identified within the primary tumor of 12 patients (14%). Five of these 12 patients had intestinal-type gastric cancer and 7 had diffuse-type gastric cancer. The stage of disease of the 12 patients who were GPT-cell positive, related to their Lauren classification, is shown in Table 2.

Presence of GPT cells within lymph node metastases

Of the 12 patients with GPT cells, 7 patients (58%) had nodal metastases, and 4 of these patients (57%) had recognizable GPT cells within these metastases (Fig. 2).

Survival

The cumulative 5-year survival for patients who had received prior acid suppression with a PPI was 60%, compared with 44% for patients who had not received acid-suppressing PPI medication (log rank 1.320; df 1; $P = 0.251$).

Table 2. Stage of disease for the 12 patients in whom GPT cells were identified, in relation to Lauren classification

	Lauren type	
	Intestinal	Diffuse
Stage		
I	1	0
II	2	3
III	2	3
IV	0	1
Early gastric cancer	1	1
Advanced gastric cancer	4	6
CLO test-positive	3	1

Fig. 1. Well-differentiated gastrin-positive tumor (GPT) cell in gastric adenocarcinoma. $\times 400$

Fig. 2. GPT cells in a well-differentiated nodal metastasis. $\times 400$

Cumulative 5-year survival was better for patients with intestinal-type gastric cancer than for those patients with diffuse-type gastric cancer (61% vs 30%; log rank 5.497; df 1; $P = 0.019$).

Prognostic significance of gastrin expression in normal gastric mucosal cells

The presence of gastrin within normal gastric mucosal cells outside the vicinity of the tumor did not influence long-term survival. Patients in whom gastrin was

identified in normal cells experienced a cumulative 5-year survival of 52% compared with a 5-year survival of 41% for patients whose normal gastric mucosa did not stain for gastrin (log rank 0.308; df 1; $P = 0.579$).

Prognostic significance of gastrin within tumor cells

The cumulative 5-year survival for patients in whom gastrin was identified within the tumor cells was 52% compared with 50% for patients without gastrin in the tumor cells (log rank 0.001; df 1; $P = 0.973$).

Fig. 3. Cumulative survival for the 86 patients in relation to presence or absence of GPT cells. Numbers at risk in boxes. Log rank 4.343, DF 1, $P = 0.037$. Continuous line, GPT cells absent; dashed line, GPT cells present

Prognostic significance of well-differentiated gastrin-positive tumor (GPT) cells

The presence of GPT cells was associated with significantly worse durations of survival. Considering the total 86 patients, the cumulative 5-year survival for the 74 patients (86%) who were GPT cell-negative was 54% compared with 30% for the 12 patients (14%) who were GPT cell-positive (log rank 4.343; df 1; $P = 0.037$; Fig. 3). Considering the 47 patients with intestinal-type gastric cancer, cumulative 5-year survival for GPT cell-negative patients was 68% compared with 0% for GPT cell-positive patients (log rank 19.917, df 1, $P < 0.001$, Fig. 4). Considering the 39 patients with diffuse-type gastric cancer, cumulative 5-year survival for GPT cell-negative patients was 33% compared with 42% for GPT cell-positive patients (log rank 0.006; df 1; $P = 0.937$). The factors found to be significantly associated with the duration of survival on univariate analysis for patients with intestinal-type gastric cancer are shown in Table 3.

Patterns of recurrent gastric cancer

A total of 33 patients (38%) developed recurrent cancer during the period of follow up. Six of the patients (50%) with GPT cell-positive tumors developed recurrent disease compared with 25 of the patients (34%) with GPT cell-negative tumors ($X^2 = 4.721$; df 1; $P = 0.278$). Local recurrence was significantly more common for patients with GPT cell-positive tumors (6 patients; 50%)

Fig. 4. Cumulative survival for the 47 patients with intestinal-type gastric cancer in relation to presence or absence of GPT cells. Numbers at risk in boxes. Log rank 19.917, DF 1, $P < 0.001$. Continuous line, GPT cells absent; dashed line, GPT cells present

Table 3. Univariate analysis of factors influencing durations of survival following R0 gastrectomy for intestinal-type gastric cancer

Factor	Log rank	df	P value
Blind acid suppression	0.06	1	0.8060
CLO test result	0.07	1	0.7979
Tumor T category	1.97	4	0.7414
Site of tumor	1.65	3	0.6491
Sex	0.29	1	0.5872
Overall stage of disease	14.47	4	0.0059
Tumor N category	12.11	2	0.0024
Presence of GPT cells	19.92	1	<0.0001
Age	67.96	22	<0.0001

than for patients with GPT cell-negative tumors (15 patients [20%]; $X^2 = 4.945$; df 1; $P = 0.026$).

Multivariate analysis

The prognostic variables entered into the model were overall stage of disease, the presence of lymph node metastases, the presence of GPT cells, and patients' ages. The presence of lymph node metastasis (hazard ratio, 2.130; 95% confidence interval, 1.198-3.787; $P = 0.010$) and the presence of GPT cells (hazard ratio, 6.609; 95% confidence interval, 1.741-25.089; $P = 0.006$) were found to be the most important predictors of survival as determined by Cox's proportional hazards model. The global X^2 for the equation was 28.502 (df 2; $P < 0.001$).

Discussion

The principal finding of this study was the identification of a new and significant prognostic indicator for patients diagnosed with intestinal-type gastric cancer. Patients diagnosed with tumors demonstrating well-differentiated cells that stained positively for gastrin had a significantly poorer prognosis than patients with GPT cell-negative tumors, irrespective of their stage of disease. The detection of GPT cell-positive tumors was unrelated to prior blind acid-suppression therapy, and overall survival was also unaffected by this practice. These findings are in keeping with those reported recently by Panter et al. [9], which suggested that although prior blind PPI therapy was associated with delays in diagnosing upper gastrointestinal cancer, this practice did not adversely affect tumor stage or long-term survival. The GPT cell phenomenon may explain why a minority of patients with apparently early gastric cancers experience poor prognoses. Stage of disease at diagnosis is acknowledged as the most important prognostic factor in gastric cancer, and 5-year survival for patients with early gastric cancer is generally very good [15]. The corrected cumulative 5-year survival for all patients with stage I or II disease in this study was 86%, and yet none of the three patients with stage I or II cancer who were GPT cell-positive survived beyond 14 months. The strength of the GPT cell phenomenon in relation to survival was further emphasized by the multivariate analysis with respect to intestinal-type gastric cancer; the GPT cell factor emerging as an independent and significant prognostic factor, despite the fact that only five patients with intestinal-type gastric cancer were GPT cell-positive.

There are several inherent limitations and potential criticisms of this study. The samples used were obtained from the histopathology archives and it is unclear what effect time-related degradation may have had on the specimens. Comparisons between specimens early and late in the case series showed similar levels and patterns of gastrin expression, providing no evidence that this was a factor. The antibody used was specific only to mature amidated gastrin and not its precursors or glycine-extended gastrin, all of which are known to have full biological activity. It is therefore possible that gastrin expression may have been underestimated. The relationship between blind prior acid-suppression therapy with powerful proton pump inhibitors (PPIs) prescribed by patients' general practitioners and survival must also be interpreted with caution. The patient cohort studied is a selected group (they had all undergone a potentially curative R0 gastrectomy) and they are certainly not representative of all patients with gastric cancer — indeed, only approximately one-third of our patients undergo potentially curative resection

[10, 11, 16]. In contrast, the study has several strengths, benefiting from robust follow-up data with accurate causes and dates of death obtained by direct inspection of patients' death certificates; over 80% of patients were followed up for at least 5 years or until death. The technique for identifying GPT cells should be available to most histopathology laboratories and, therefore, reproducing these results is not anticipated to be a problem. Furthermore, the patients were recruited from a consecutive series of patients diagnosed with gastric cancer from one geographical region and all had been treated by the same specialist surgeon using a standardized operative technique without the confounding variable of neoadjuvant chemotherapy.

The evidence for gastrin as a trophic agent for gastric cancer is abundant [3]. Certain gastric cancers possess both low- and high-affinity gastrin/CCK-B receptors and respond to pharmacological, and on occasion, physiological concentrations of gastrin [17, 18]. In addition, gastrin-releasing peptide (GRP) has been shown to contribute to the growth of gastric cancer *in vitro* and in human gastric cancer models [19]. Given that PPI therapy induces hypergastrinemia, it is perhaps surprising that there is no evidence that this influences outcomes for patients diagnosed with gastric cancer. A possible explanation may be that gastrin's trophic effect is mediated by autocrine or paracrine means, and systemic gastrin levels may be irrelevant. Certainly, studies in colorectal cancer have shown low gastrin levels within the tumor and increased circulating nonamidated gastrin, *i.e.*, only partially processed gastrin, suggesting that the tumor may be secreting gastrin with resulting hypergastrinemia [20]. This theory is supported by reports of reductions in serum gastrin levels after resection of primary colorectal tumors [21, 22] although conflicting evidence exists [23, 24]. Serum gastrin measurement may be unreliable for two reasons: commercial antisera react with amidated gastrin, and as nonamidated gastrin is the fully active biological entity, the results of such assays are suspect. Disruption of the feedback mechanism for gastrin release induced by PPI therapy produces not only fasting hypergastrinemia but also even greater postprandial hypergastrinemia. Normal practice measures the level of fasting gastrin, but the degree of hypergastrinemia may be underestimated, and studies into the influence of hypergastrinemia on outcomes for patients diagnosed with gastric cancer should be interpreted in light of the above limitations.

Gastrin receptor antagonists have been reported previously to have an inhibitory effect on the development and progression of both gastric cancer [25, 26] and colorectal cancer [27], both in animal models and *in vitro*, but early clinical studies were disappointing [28]. Whether GPT cells have a trophic influence on tumors or are simply a marker of another unidentified mediator

is unclear. Certainly, the presence of GPT cells correlated significantly with the presence of gastrin within tumor cells in general, and arguably GPT cells are producing gastrin with potential local autocrine or paracrine effect. This hypothesis is supported by the finding that patients with GPT cell-positive tumors were significantly more likely to develop local recurrent cancer. Such patients may in future benefit from the development of specific gastrin receptor antagonists, and it is on these specific patients that further work should be focused.

In conclusion, the proliferative effects of GI hormones on neoplastic tissues are well described. The findings of this study have several potentially important implications for the study of gastric cancer, both with respect to understanding the pathophysiology of the disease and also in the development of new therapies. GPT cells were identified in 14% of all gastrectomy specimens and in 11% of intestinal-type tumors. Histopathologically, GPT cells resembled normal antral G cells, yet their gastric anatomical location varied widely, and their origin is uncertain. The relationship between GPT cell-positive tumors and survival was confined to patients with intestinal-type gastric cancer, and gastrin's trophic effect on human gastric cancer is also known to be confined to this cancer subtype [4]. Recent reports have associated *Helicobacter pylori* infection with increased gastrin [3], and as *H. pylori* is a recognized risk factor for gastric cancer, this relationship deserves further investigation. Gastrin receptor antagonists have variable effects on gastrin-stimulated growth, but prostaglandin derivatives have been reported to inhibit the growth of gastrin receptor-positive cancers, whereas they have no effect on receptor-negative cancers [10]. Future research should be directed towards assessing gastric cancer hormone receptor status, as with other site-specific tumors, so that specific gastrin antagonists may be developed to facilitate possible adjuvant chemotherapy for subgroups of patients diagnosed with gastric cancer.

Acknowledgments The histopathological support and advice provided by Professor Geraint Williams and Dr. Jasani of the Department of Pathology, University Hospital of Wales, Cardiff, is gratefully acknowledged.

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