Loss of Heterozygosity of Chromosome 1q in Gastrinomas: Occurrence and Prognostic Significance

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ABSTRACT

A proportion of gastrinomas demonstrates aggressive growth, and most deaths occur in this group. Little is known about the molecular pathogenesis of this tumor, and there are no predictive factors that are useful in an individual patient. Chromosome 1 (Chr 1) loss of heterozygosity (LOH) is frequent in a number of nonendocrine tumors and in a few endocrine tumors, and its presence can correlate with tumor aggressiveness and survival. In gastrointestinal endocrine tumors including gastrinomas, little data are available on Chr 1 LOH, and the limited results are contradictory. In the present study we determine whether Chr 1 LOH occurs in gastrinomas and is associated with aggressive growth by performingChr 1 allelotyping with microsatellite markers in microdissected tumor tissue from 27 human gastrinomas and the leukocyte DNA of the patients. Detailed clinical pathological correlations were possible, because tumor growth in all of the patients was prospectively assessed with yearly imaging studies. Twelve gastrinomas (44%) had Chr 1 LOH, and in all of the cases 1q LOH occurred. 1q LOH was associated with aggressive growth (P = 0.0004), presence of liver metastases (P = 0.019), and postoperative development of hepatic metastases (P = 0.017). Eight (75%) of the 12 tumors with 1q LOH had 1q31–32 LOH over a 17.3 cM region, whereas LOH in 6 tumors (50%) occurred at 1q21–23 over a 12.3 cM area. The presence of 1q31–32 LOH and 1q21–23 LOH correlated with aggressive tumor growth (P = 0.0056 and P = 0.0031, respectively), and with postoperative development of liver metastases (P = 0.0114 and P = 0.011, respectively). These data suggest that 1q LOH is not infrequent in gastrinomas and could be a molecular/genetic prognostic factor for aggressive growth that could be useful clinically. The high frequent allelic loss at 1q31–32 as well as 1q21–23, which was associated with tumor aggressive growth, suggests these two regions harbor putative tumor suppressor gene(s) that are important for aggressive growth of this tumor.

INTRODUCTION

PETs, similar to carcinoid tumors, are generally slow growing (1). However, recent studies report that a proportion of these tumors demonstrate aggressive growth with shortened survival (1–5). Unfortunately, in an individual patient there are no clinical, laboratory, or tumor histological features that reliably predict which PET will pursue an aggressive course (1). If prognostic factors could be identified that accurately predicted the growth behavior of PETs in an individual patient, more aggressive surgery or antitumor treatment could be started earlier in the subset with a poor prognosis. Furthermore, the molecular mechanisms responsible for the development and/or progression of PETs are largely unknown. Alterations of common oncogenes (i.e., ras, myc, and src) or tumor suppressor genes such as p53 or the Rb gene in typical PETs are not common (6). Recently, mutations in the MEN1 tumor suppressor gene on 11q13 were reported to occur in 16–40% of sporadic PETs and inactivation of p16INK4a/CDKN2A to occur by either methylation of 5′-CpG islands in the gene promoter (50–52% of gastrinomas) or homozygous deletions of the gene (0–40%; Refs. 6–9). However, neither MEN1 gene mutations nor inactivation of the p16INK4a/CDKN2A gene in gastrinomas correlated with aggressive growth (6–8).

A number of studies showed recently that Chr 1 LOH is frequent in various nonendocrine tumors (breast, pancreatic, gastric, and colon; Refs. 10–13) and in a few endocrine tumors (14–18), and the presence of Chr 1 LOH correlates with decreased survival (19–22), increased tumor aggressiveness (23), and the presence of metastatic disease (14). However, the studies in carcinoids and PETs including gastrinomas have given contradictory results (14, 16, 24–26). Moreover, the results are frequently limited because of the few cases, few loci examined, and lack of long-term follow-up in the patients to determine the growth pattern of the tumor (14, 16, 18, 26–30). The latter point is particularly important, because a recent study shows that in only 40% of patients with hepatic metastases does the tumor demonstrate aggressive growth, and all of the deaths occurred in this subset (4). Therefore, the purpose of the present study was to determine whether Chr 1 LOH occurs in gastrinomas, and if LOH occurs, to define the narrowest area where the highest frequency occurs and to correlate the presence of Chr 1 LOH with tumor growth behavior as well as clinical features.

MATERIALS AND METHODS

Patients, Tumors, and Clinical Variables. Twenty-seven patients with ZES evaluated at the NIH between 1989 and 1998 were included in this study. The study protocol was approved by the Clinical Research Committee of the National Institutes of Diabetes, Digestive and Kidney Diseases, and all of the patients gave informed consent. The diagnosis of ZES was established as reported previously (31, 32). Preoperative fasting gastrin, secretin (2 clinical units secretin/kg), and calcium (5 mg/kg of calcium × 3 h) gastrin provocation tests were performed as described previously (31). Conventional imaging studies (computed tomography with oral and i.v. contrast, magnetic resonance imaging, ultrasonound, and bone scan), selective abdominal angiography with secretin stimulation and hepatic vein gastrin sampling, and SRS were performed (33, 34) to locate the primary tumor and evaluate the extent of disease. Each patient underwent an exploratory laparotomy for attempted curative resection (33). Patients were reassessed postoperatively to determine disease-free status and annually to monitor progression of disease (35). Disease-free status was defined by normal fasting serum gastrin levels (<200 pg/ml), negative results on gastrin provocative testing with secretin (<200 pg/ml increase) and/or calcium (<395 pg/ml increase), and no evidence of tumor on any imaging study (31, 33, 35). In those patients that were not disease-free postoperatively, annual detailed imaging studies (computed tomography, magnetic resonance imaging, ultrasonound, and SRS) and, if the results were unclear, selective angiography provided the basis for assessment of tumor growth or progression (4, 8). Consistent absence of imaged lesions or lack of increase in size or number of lesions over the follow-up period was defined as a tumor not demonstrating growth. An increase in size or number of lesions on imaging studies was defined as evidence of tumor growth as described previously (4). For each patient the number and size of each measurable tumor were determined in transverse sections of an imaging modality. The measurements were performed with a caliper on a hard copy image using an accompanying scale.

Received 7/18/02; accepted 12/16/02.

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2 The abbreviations used are: PET, pancreatic endocrine tumor; LOH, loss of heterozygosity; Chr 1, chromosome 1; ZES, Zollinger-Ellison syndrome; MEN1, multiple endocrine neoplasia type 1; SRO, shortest region of highest overlap; MSI, microsatellite instability; SRS, somatostatin receptor scintigraphy.

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The volume of the tumor was calculated using $V = \frac{4}{3}\pi r^3$, where $r$ is the radius of the tumor. The growth rate was calculated as the percentage of volume increase per month over the time of the study. The rate of change in the most rapidly growing hepatic or extrahepatic tumor or initial disease extent was used to determine the growth category. Patients were stratified into two groups based on tumor growth rate or tumor extent. Patients were classified as having a nonaggressive form of ZES if there was no growth or a <25% increase in volume per month either with or without liver metastases at all of the yearly evaluations and if initially there was not extensive metastatic disease. Patients were classified as having an aggressive form of ZES if there was an increase in tumor growth of ≥25% in volume per month, the appearance of a new lesion(s) at any follow-up evaluation, or if there was initially extensive metastatic disease. The development of liver metastases identified by imaging studies during follow-up served as the definition for patients who developed liver metastases (3, 4).

Liver metastases were confirmed by percutaneous liver biopsy.

**DNA Samples.** Tumor tissue of 27 patients with sporadic or MEN1-associated gastrinomas was obtained by microdissection from frozen 5-μm sections. Tumor genomic DNA was obtained by 3 days of digestion of tumor tissue in 30 μl of 10 mM Tris (pH 8.0)-1 mM EDTA solution (Tris 10 mM, EDTA 1 mM, protease K 0.5 mg/ml, and Tween 20 0.5%) at 37°C. Normal control genomic DNA was isolated from peripheral blood leukocytes from each patient by using the PUREGENE DNA isolation kit (Gentra System, Minneapolis, MN). DNA was stored at 4°C until PCR amplification.

**Microsatellite DNA Markers of Chr 1.** Thirty-seven loci spanning the whole of Chr 1 were detected by using primer pairs for 38 di- and tetranucleotide repeat markers (Fig. 1), which were ordered from Research Genetics (Huntsville, AL) based on the Genome Database.

**PCGR.** The template DNA of both tumor and leukocytes was amplified by PCR using a GeneAmp PCR system 9700 Thermocycler (PE Applied Biosystems, Foster City, CA) with the following conditions: 94°C for 5 min, 94°C for 45 s, annealing at 59°C for 45 s, and extension at 72°C for 45 s for a total of 32–35 cycles. Each reaction contained 1.5 μl of 10× PCR buffer, 1.5 μl of tumor DNA or normal control DNA (approximately 10–20 ng), 10 pmol of each primer, 2.5 units of Gold Taq polymerase (Applied Biosystems), and 130 μM each of deoxynucleoside triphosphate. The final concentration of MgCl2 was 1.5 mM. [α-32P]dCTP (ICN Biomedicals, Inc., Costa Mesa, CA; >3000 Ci/mmol) was also included in each PCR reaction (1 μCi/reaction).

**Analysis of Allele Loss.** After PCR, products were mixed with loading dye (v/v = 1:1), denatured at 95°C for 8 min, then chilled immediately to 4°C. Four or 5 μl of the product was analyzed by electrophoresis through a 6 or 8% denaturing polyacrylamide gel (Invitrogen, Carlsbad, CA) in 1× Tris-borate EDTA buffer. The gels were then dried and exposed to X-ray films (Kodak X-OMAT AR; Eastman Kodak Company, Rochester, NY) with or without an intensifying screen for 8 h–3 days. The intensity of signals in the films was analyzed by using Kodak Image station 440 system (Eastman Kodak Company). LOH was defined as a reduction of at least 50% in the allelic ratio between the tumor and normal DNA from the same patient (18, 19).

**Statistical Analysis.** Significance was calculated using the Mann-Whitney test and Fisher exact test. $P < 0.05$ were considered significant.

**RESULTS**

Twenty-seven gastrinomas from 27 different patients were studied. The average age of the patients at surgery was 45.5 ± 2.1 years; 44% were males, and the duration of the disease before surgery was 8.3 ± 1.3 years, which is similar to most surgical series of patients with ZES (32, 33). Only 4% (1 of 27 patients) had MEN1 with ZES, which is less than the 20–25% seen in most series of patients with ZES (32, 36), because in our protocol only patients with ZES with tumors >2.5 cm undergo surgery (33). Most patients had a markedly elevated fasting gastrin level (mean, 4,140 pg/ml; range, 87–1,100,000 pg/ml) and acid secretory rates (mean basal acid output, 42 ± 4 mEq/ h), which is characteristic of ZES (36). Similar to other recent surgical series (33, 36), the most common primary tumor site was the duodenum (48%), being twice as frequent as pancreatic gastrinomas (26%) and 3.5 times as frequent as gastrinomas in other sites (18%). The disease-free rate was 63% immediately postresection, and it decreased to 44% during the mean 7-year follow-up, which is similar to other surgical series (33). During the follow-up, in 41%
of the patients the gastrinomas showed growth and liver metastases developed in 26%.

In the gastrinomas from each of the 27 patients, possible LOH in 37 loci spanning the whole Chr 1 was investigated by using 38 di- or tetrapolymorphism markers spaced throughout the chromosome (22 for 1p, and 16 for 1q; Fig. 1). All of the loci assessed were informative in >66% of the gastrinomas. One locus in 1q 31–32 was tested by two markers, D1S245 and D1S471, because each of two markers showed low informative of <50% when it was used alone. Informative data from each individual tumor was >75% (Fig. 1). Chr 1 LOH was identified in 12 of 27 gastrinomas (44%). All of these 12 tumors had 1q LOH (44%). LOH was found in 1q only in 4 tumors (15%), and LOH occurred in both arms in 8 tumors (30%; Fig. 1). The frequency of LOH with different markers varied from 0 to 23% (Fig. 1). The most frequent LOH was seen with markers D1S1667 and D1S2796 at 1q31–32 with a fractional ratio of LOH of 23 and 22%, respectively (Fig. 1), followed by D1S1677 at 1q23.2 and D1S2715 at 1q21 with a fractional ratio of LOH of 19 and 18%, respectively (Fig. 1). Of the 12 patients with gastrinomas showing Chr 1 LOH, in 9 tumors LOH was detected in >3 different loci (Fig. 1). Representative results of LOH at different Chr 1 loci are shown in Figs. 1–3. Patient #5 had LOH in almost every locus in Chr 1 in both 1p and 1q (Figs. 1–3), and patient #22 had both LOH and MSI throughout the whole Chr 1 (Fig. 1). Patient #16 lost approximately half of 1p (Figs. 1–3). Patient #17 had LOH throughout almost all of 1q (Fig. 1). Three patients (#2, 10, and 19) had only 1 locus of LOH on Chr 1, and in each case it was in 1q (Figs. 1 and 2).

In a number of different tumors, both endocrine and nonendocrine (11, 12, 14, 19, 21, 37), LOH in Chr 1 has been correlated with tumor growth, aggressive tumor behavior, or tumorigenesis. To determine whether similar correlations might exist in gastrinomas, the presence or absence of Chr 1q LOH was correlated with various clinical, laboratory, or tumor variables reported to be associated with gastrinoma growth behavior or aggressive growth (1, 3). Of the 12 tumors with 1q LOH, 10 tumors showed an aggressive growth pattern after resection (83%), whereas this occurred significantly less frequently ($P = 0.0004$) in tumors without 1q LOH (13%), with only 2 of 15 tumors (Table 1) showing aggressive tumor growth behavior. 1q LOH was identified in 8 of 12 tumors (67%) with hepatic metastases, whereas only 3 of 15 tumors (20%; Table 1) without liver metastases demonstrated 1q LOH ($P = 0.019$). During the mean 7 years of follow-up postresection, 7 patients postoperatively developed hepatic metastases, and tumors from 6 of these patients had 1q LOH (86%), whereas only 6 of 20 tumors (30%) from the 20 patients who did not develop postoperative hepatic metastases had 1q LOH ($P = 0.0165$). Nonduodenal gastrinomas, which are reported to show more aggressive growth than duodenal tumors (1, 3, 5), demonstrated 1q LOH in 6 of 10 tumors (60%), which was a comparable frequency to duodenal tumors, which had 1q LOH in 5 of 14 (36%; $P = 0.22$; Table 1). No significant correlation was observed for the presence or absence of 1q LOH with age, gender, primary tumor size, tumor volume, postoperative cure rate, either short- or long-time acid secretory rates, or...
fasting serum gastrin levels (Table 1). Furthermore, in previous studies, gastrinomas have been shown to frequently have p16INK4a/CDKN2A gene abnormalities (6, 8, 9), and up to one-third of sporadic gastrinomas have been shown to possess MEN1 gene mutations (1, 6). The presence or absence of either of these gene abnormalities did not correlate with the presence or absence of 1q LOH (Table 1).

In the present study, 12 tumors had LOH detected somewhere in Chr 1q; however, 8 of these 12 tumors (75%) had LOH at 1q21–31. The SRO is defined by a 17.3-cM interval (SRO1) flanked by the markers D1S158 and D1S1667 (Fig. 1). Another distinct interval of LOH was determined at 1q21.3–23.2; of 12 tumors with chromosome 1q LOH, 6 tumors (50%) had LOH in this SRO, a region of 12.3 cM (SRO2) flanked by D1S2715 and D1S1640 (Fig. 1). Seven of the 8 tumors with SRO1 LOH showed an aggressive growth pattern after resection (87.5%), whereas only 5 of 19 tumors without SRO1 LOH (26.3%) showed an aggressive tumor growth pattern (P = 0.0056; Table 2). In the 8 tumors with SRO1 LOH, 5 patients developed liver metastases postoperatively (62.5%), whereas only 2 of 19 tumors (11%) without SRO1 LOH developed new hepatic metastases (P = 0.011). Similarly, in 6 of 7 tumors (100%) with SRO2 LOH, a postoperative aggressive growth pattern was seen, whereas only 6 of 21 tumors (28.6%) without SRO2 LOH showed an aggressive growth pattern (P = 0.0031). Four of the 6 tumors with SRO2 LOH postoperatively developed liver metastases (66.7%), whereas only 2 of 21 tumors (9.5%) without SRO2 LOH developed hepatic metastases after resection (P = 0.011; Table 2).

Table 2. Comparison of aggressive tumor growth and postoperative development of liver metastases with the SRO of LOH chromosome 1 (SRO1 or SRO2)

<table>
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<th>Characteristic</th>
<th>SRO-1 LOH</th>
<th>SRO-2 LOH</th>
</tr>
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<tbody>
<tr>
<td>Liver metastases postoperatively</td>
<td>6 (26.3%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Aggressive tumor growth</td>
<td>7 (37.5%)</td>
<td>5 (26.3%)</td>
</tr>
</tbody>
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The 17.3-cm interval on 1q flanked by markers D1S518 and D1S1667 (Fig. 1) in which 8 of 12 tumors with chromosome 1q LOH demonstrated LOH. SRO2 is a 12.3 cM region of 1q flanked by D1S2715 and D1S1640 (Fig. 1) in which 6 of 12 tumors with 1q LOH have LOH (Fig. 1).

### DISCUSSION

Relatively little is known about the molecular pathogenesis of gastrinomas or other malignant PETs (6, 29). Alterations of common proto-oncogenes (i.e., ras, myc, and src) or common tumor suppressor genes, such as p53 or the Rb gene in typical PETs, are not common (6). Mutations in the 10-exon MEN1 tumor suppressor gene located at 11q13 were found recently in 27–39% of sporadic gastrinomas (6, 7). Furthermore, alterations in the p16INK4a tumor suppressor gene are reported in gastrinomas, with hypermethylation of 5′-CpG islands in 50–52% (6, 8, 9), and in one study (9) 42% of the gastrinomas had homozygous deletions of p16INK4a/CDKN2A gene, whereas another (8) showed no homozygous deletions. An increase in the HER-2/neu gene product is reported in 0–17% of PETs (6, 9, 38). However, these alterations occur in only a subset of PETs and have not been shown to have prognostic significance (6–8).

Insights into the molecular determinants of the development and progression of PETs are important for a number of reasons. Except for insulinomas, the majority of PETs are malignant (39). Recent studies demonstrate that the primary determinant of long-term survival in patients with PETs is now the natural history of the tumors per se rather than excess hormone production by a functional tumor as it was in the past (1, 2, 39). With gastrinomas, recent studies demonstrate that 15–30% pursue an aggressive malignant course, whereas the remainder have an indolent course (1, 3, 5). Furthermore, even among patients with metastatic PETs to the liver, only a subset shows a malignant course, and all of the deaths occur in this subset (4). At present, no clinical or laboratory parameters have been identified that are sufficiently predictive to be clinically useful in determining the subsequent growth pattern of the tumor in an individual patient to allow early institution of antitumor treatments (1, 6). Furthermore, studies of the limited altered molecular factors identified in these tumors have shown that they are not generally predictive of the biological behavior of these tumors (6–8, 38).

Recently, studies in nonendocrine tumors (10, 11, 13, 20, 37, 40–44) and a few endocrine tumors (14–18) report that Chr 1 LOH is found frequently, and its presence can be associated with decreased survival (19–22, 37), increased tumor aggressiveness (11, 23, 43, 45), and the presence of metastatic disease (12, 14, 44, 46). Studies in carcinoids and PETs including gastrinomas have given contradictory results (14, 16, 24–26). Moreover, the results are limited for a number of reasons. Studies of Chr 1 LOH in PETs and carcinoids were often limited by inclusion of only a few cases, only small numbers of loci in Chr 1 were examined, microdissected tumor tissue was usually not used so the frequency of Chr 1 LOH could be underestimated, and most important, long-term follow-up was not frequently done to assess the malignant behavior of these frequently slow-growing tumors to allow correlation with the Chr 1 findings (14, 16, 18, 26–30).
IQ LOH IN GASTRINOMAS

Therefore, the purpose of the present study was to determine whether Chr 1 LOH occurs in gastrinomas, its frequency, and if LOH occurred, to correlate the presence of Chr 1 LOH with tumor biology and tumor growth behavior. This study had none of the limitations of many previous studies outlined above. It includes a significant number of cases to allow correlations (i.e., 27 cases), many loci (37 loci) spread evenly throughout Chr 1 were investigated, tumor tissue was obtained by microdissection, and all of the patients were part of a prospective study assessing tumor growth, with yearly evaluations with multiple imaging studies allowing correlations with tumor growth rate.

We found that 12 of 27 gastrinomas (44%) from 27 different patients had Chr 1 LOH, and in all of the cases 1q LOH was found. This finding is much higher than the rate of 9–25% from other studies of various PETs including gastrinomas reported in three studies using similar methodology to ours (14, 16, 27) but similar to the frequency of 38% for Chr 1 LOH reported in a study of 8 gastrinomas (29) and the 44% reported in a study of nonfunctional PETs (24). The higher frequency of Chr 1 LOH in gastrinomas and/or PETs in our study and two other studies (24, 29), than the three earlier studies cited above is likely related to the number of informative microsatellite markers used in each study. In our study we used 38 microsatellite markers for 37 highly polymorphic loci with each being informative in >67% such that informative data in each patient was >75%. In our study and the previous two reports (24, 29) reporting a 38–48% frequency of Chr 1 LOH, 31–47 markers were used, whereas in the three studies (14, 16, 27) showing a lower frequency of LOH, only 2–12 markers were used. Furthermore, microdissection, a method that could eliminate the contamination of normal tissue as well as fibrosis tissue, was not frequently used in the studies showing a low frequency of LOH (14, 27, 28).

A number of studies on other endocrine tumors (pheochromocytomas, thyroid tumors, and parathyroid tumors) as well as neuroblastomas, which share some structural features with PETs and a number of nonendocrine tumors (pancreatic, hepatic, gastric cancer, and meningiomas), also showed a 32–50% frequency of Chr 1 LOH (11, 13, 17, 18, 40, 42) similar to our results (44%). Other studies on pheochromocytomas and neuroblastomas, as well as studies on some nonneuroendocrine tumors (cervical carcinomas) reported an even higher frequency of Chr 1 LOH (61–93%; Refs. 22, 43) than we found in gastrinomas. However, some studies on endocrine tumors (parathyroid tumors, thyroid tumors, and neuroblastomas) as well as nonendocrine tumors (pancreatic cancer, endometrial carcinomas, and breast cancer) reported a relatively low frequency of Chr 1 LOH (18–30%) compared with our data (19, 20, 23, 45). These results support the conclusion that in contrast to the lack of alterations of common tumor suppressor genes (p53 and Rb) and common oncogenes (myc, src, and so forth) in PETs/carcinoids and other endocrine tumors (6), Chr 1 LOH is not uncommon in these tumors, similar to results in a number of nonendocrine tumors.

A number of studies report that the presence of Chr 1 LOH has prognostic value in different nonendocrine tumors (breast, gastric, pancreatic, colorectal, gastrointestinal smooth muscle tumors, and meningiomas) and some neuroendocrine tumors (neuroblastomas and thyroid carcinomas; Refs. 11, 14, 19–23, 46, 48). In nonendocrine solid tumors the presence of 1p LOH correlated with increased postoperative recurrence of tumors (23), advanced tumor stage (43, 45), and decreased survival (46, 48). In patients with neuroblastomas, several studies show that if 1p LOH is present, the patients have a shortened survival (20–22). In patients with papillary thyroid carcinomas, a higher frequency of 1q LOH was associated with decreased survival (19). In our study there was a highly significant (P = 0.0004) correlation with the presence of Chr 1q LOH and aggressive tumor growth.

A number of studies in PETs and gastrinomas have reported that different clinical and laboratory valuables are associated with aggressive tumor growth including the presence of hepatic metastases, decreased survival, large primary tumor size, nonduodenal primary tumor location, female gender, and high levels of various tumor markers (1–3, 5, 36). Our data showed that gastrinomas with hepatic metastases have Chr 1q LOH more frequently (67%) than the tumors without hepatic metastases (20%; P = 0.019) and that 1q LOH was more frequently present in gastrinomas of patients developing liver metastases postoperatively (P = 0.017). Our data are consistent with the proposal by some (14) but not by others (24) that liver metastases may be more frequent in PETs with Chr 1 LOH. Although nonduodenal gastrinomas have been shown to have a more aggressive growth pattern (1, 3, 5), their presence did not correlate with chromosome 1q LOH. Neither our results nor studies on other tumors (gastrointestinal smooth muscle tumors, breast, lung, stomach, kidney, ovary, endometrium, colorectal cancer, and PETs) show that the frequency of Chr 1 LOH is not uncommon in these tumors, similar to the frequency of various oncogenes or tumor suppressor genes located in other chromosomes. For example, with neuroblastomas, Chr 1 LOH correlated with MYCN amplification as well as loss of expression of somatostatin receptors in tumors (49). However, our data did not show a correlation of Chr 1 LOH with the presence of alterations in the tumor suppressor gene MEN1 or p16INK4a/CDKN2A, or with the expression of somatostatin receptors in gastrinomas. All of the gastrinomas in the present study were visualized with SRS demonstrating high levels of somatostatin receptor subtypes 2 and/or 5. Because very few factors have been identified in patients with gastrinomas that are predictive of postoperative growth in an individual patient (1), any such factor could be clinically useful. Therefore, our findings in this study demonstrate that Chr 1 LOH correlates with aggressive growth and the presence of hepatic metastases, which has been shown to be one of the most important predictors of long-term survival in these patients (1–5, 36), suggesting that assessing Chr 1q LOH in gastrinomas could be useful in their clinical management.

Previous surveys on Chr 1 demonstrated that 1p32 LOH (29–36%; Refs. 17, 22) and 1p35 LOH (8–46%; Refs. 11, 13, 18, 41) are not infrequent in many nonendocrine and endocrine tumors. Furthermore, numerous studies on endocrine and nonendocrine tumors show frequent allelic loss (18–45%) in 1p36 (20, 23, 27, 40, 42, 43, 49); thus, this area was proposed to potentially harbor an important tumor suppressor gene(s; Refs. 40, 42). In our study, 8 of 27 gastrinomas had LOH on 1p (30%); however, only 5 tumors had LOH at 1p36 (19%) as well as 3 tumors with LOH at 1p32 (11%). In contrast, 44% had 1q LOH. These data suggest that 1q LOH likely plays a more important role in the pathogenesis of gastrinomas than 1p LOH. A number of previous reports show higher frequency of 1q LOH than 1p LOH in PETs, carcinoids, thyroid carcinomas, and medulloblastomas (18, 19, 27). In our study the SROs were at 1q31–32 (SROI) and 1q21–25.1 (SRO2). Eight of 12 (75%) Chr 1 LOHs occurred at 1q31–32 over a 17.3-cM region, whereas 6 of 12 (50%) Chr 1 LOHs occurred at 1q21–23 over a 12.3-cM area. The presence of LOH at SRO-1 as well as SRO-2 correlated with aggressive growth of the gastrinomas and was significantly associated with the postoperative development of hepatic metastases. These results suggest that 1q31–32 as well as
1q21–23 harbor genes that contribute to aggressive growth of gastrinomas or possibly to development of hepatic metastases. Our study is the first to identify this region as harboring important genes affecting growth behavior for PETs and/or carcinoids. One recent study (50) reported that the HPT-JT locus was within a 0.7-cM region on 1q25–q31, which overlapped with the SRO-1 in our study. Studies on anaplastic thyroid carcinomas and medulloblastoma showed a frequency of 40% LOH at 1q32–43 (18) and 36% LOH at 1q31–32 (51). At present it is unknown which tumor suppressor genes in 1q31–32 or 1q21–23 are playing a role in determining aggressive growth behavior as well as contributing to the development of hepatic metastases in gastrinomas. However, tumor suppressor gene 

MDA7/IL-24, which induces apoptosis in human lung, pancreatic, breast, colorectal, and prostate cancer cells, as well as melanoma cells (52) was localized between locus DIS306 and DIS491 (215.2 cM-227.8 cM), which exactly overlaps with our SRO-1 region (53). Human malignant melanoma metastases suppressor gene KiSS1 (54) is also localized at 1q32 close to DIS504 (218.5 cM; Ref. 55), which is within our SRO-1 area. Additional study involving additional tumors will be needed to narrow these two regions to identify which genes may contribute to the hepatic metastases or aggressive growth in sporadic gastrinomas, as well as to confirm the findings of our study.

In conclusion, our study demonstrates that Chr 1 LOH occurs in almost one-half of gastrinomas, and in all of the cases it was present in 1q. The presence of 1q LOH correlated significantly with aggressive growth of gastrinomas and the development of hepatic metastases. The SRO for LOH at 1q31–32 and 1q21–23 also demonstrated a significant correlation with aggressive growth and the development of hepatic metastases. The results should be useful in at least two ways. First, assessment of 1q LOH can be useful as a molecular prognosticator to identify the subset of patients whose tumors show aggressive growth and/or develop hepatic metastases. Second, the definition of two regions of SRO in Chr 1q can serve as a starting point for future studies to identify which genes may be responsible for this growth behavior in these tumors.

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