Survey of Genetic Alterations in Gastrinomas

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ABSTRACT
Gastrinomas are rare gastrin-secreting endocrine tumors that usually arise in the duodenum or pancreas and, if untreated, can cause severe peptic ulcers or metastatic disease. Although most tumors are sporadic, they are especially common in patients with multiple endocrine neoplasia type 1 (MEN1), and most studies of these tumors have focused on the role of the MEN1 gene. Although the gene is commonly altered in sporadic tumors, this finding is not universal, and it is highly likely that other genetic defects play a significant role. In the present study, an in-depth analysis of the DNA of eight tumors was carried out in an effort to localize these areas. The experiments consisted of an analysis of 400 microsatellite marker loci distributed evenly throughout the human genome, and the results were confirmed with comparative genomic hybridization. Whereas deletions encompassing the MEN1 gene were seen in two tumors, the most striking result was multiple large rearrangements on chromosome 1 in two of the tumors with hepatic metastases. In several instances, an individual tumor had abnormalities of every informative maker on a given chromosome, presumably as a result of aneuploidy affecting that chromosome. Such defects were only seen in the four large or aggressive tumors, and the total number of chromosomes affected in a tumor ranged from 1 to a high of 13 in a patient who had an unusually aggressive tumor. This tumor also showed microsatellite instability, and this is the first report of such a defect in gastrinomas. This study implicates chromosome 1 defects, aneuploidy, and perhaps mismatch repair defects as important features of gastrinomas; deletions involving the MEN1 gene were confirmed, but the rest of the genome was free of large deletions or amplifications.

INTRODUCTION
Gastrinomas are rare tumors that usually arise in the duodenum or pancreas and often metastasize to the lymph nodes or the liver. They are considered a subset of endocrine tumors and, as a result of their secretion of the hormone gastrin, cause virulent peptic ulcer disease known as the Zollinger-Ellison syndrome. All patients gave informed consent in a protocol approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board. The criteria for diagnosis of the Zollinger-Ellison syndrome, the MEN1 syndrome, and gastrinoma used by the Zollinger-Ellison study group have been well described in recent reports (1–3) and consisted of a detailed clinical evaluation and histological examination with a positive immunostain for gastrin. Seven patients had sporadic gastrinomas, and one patient had a MEN1-associated gastrinoma. Three of the patients had aggressive tumors in that the tumor burden or growth was considered life-threatening. Other selected characteristics of the patients are given in Table 1. All tumors were frozen in liquid nitrogen at the time of surgery and stored with paired blood samples at −70°C. One sample (30–100 mg in size) from a discreet tumor focus was used from each patient. All tumor samples were examined histologically and judged to consist almost entirely of tumor cells (>80%), without significant foci of nonmalignant cells or normal adjacent tissue, and microdissection was not used to enrich for tumor cells.

DNA Preparation. Genomic DNA from tumor tissue and blood was extracted using the QIAamp system (Qiagen, Inc., Valencia, CA). An additional phenol extraction step was found to be useful in increasing the yield of subsequent PCR amplifications. DNA concentrations were determined spectrophotometrically, and all samples were examined by agarose gel electrophoresis to confirm that the DNA was at least several hundred bp in length and thus not significantly degraded.

PCR and Product Analysis. The PCR amplification and analysis were modified from a protocol used in automated genotyping experiments to robustly amplify microsatellite markers (24). The polymorphic markers used included all 387 markers in the Cooperative Human Linkage Center Screening set (Weber Version 8), and the fluorescence-labeled primers were purchased from Research Genetics, Inc. (Huntsville, AL). These markers were selected because of their even distribution throughout the whole human genome and because of their high information content with an average heterozygosity of 76%. Subsequent experiments used 23 markers distributed throughout chromosome 1 from the ABI PRISM Linkage Mapping Set (Perkin-Elmer) and 4 markers from the MEN1 region on chromosome 11q. Two of these markers, D11S134 and D11S1357, were from Perkin-Elmer, and the other two, PPP1CA (25) and PYGM (26), were synthesized.
RESULTS

Tumor Characteristics. A selected summary of the eight tumors analyzed is given in Table 1. Patients 2, 3, and 7 had large, aggressive tumors, and the growth of these tumors was the direct cause of significant morbidity, independent of the tumors’ production of gastrin. Patient 6 had a large but slowly growing tumor that was a manifestation of the MEN1 syndrome, and in the other patients, the tumors were presumably of sporadic origin. All but one patient had documented metastases to lymph nodes, and three patients had hepatic metastases.

Nature of the Data and Information Obtained. The use of fluorescent primers in the PCR reaction and an automated sequencer facilitated the quantitative analysis of the intensity of each marker allele. An example of a trace of an individual marker showing no abnormality is shown in Fig. 1A. The two alleles from normal blood DNA from a patient are shown above the trace from the patient’s tumor DNA. Generally, the amount of PCR product from the normal DNA was slightly less than that from the tumor, and the larger-sized allele on the right was amplified slightly less well than the smaller-sized allele on the left; however, the overall appearance of the traces are very similar. A simple way to summarize such a result was first used in a genetic analysis of gastric cancer (30). Quantitatively, the ratio of the intensity of peak a to peak b is very close to the ratio of peak c to peak d. An alternative way of expressing this observation is that the value of (aalb)/(cdl) is close to unity. The term AI has been used to describe the condition in which this value is not close to unity.

Fig. 1B shows LOH presumably resulting from a deletion encompassing one allele in the tumor DNA. A small residual peak remains from the nonmalignant cells in the sample. Fig. 1C shows a more subtle form of AI. Although the result is not as dramatic as LOH, the degree of AI was more than 2 SDs from the mean, and such results were often present in multiple adjacent chromosomal markers. This result is probably due to a duplication of the allele on the right. An alternative explanation is that the allele on the left was deleted in about half of the tumor cells, but this is less likely because the DNA from each tumor was extracted from an individual focus, and the tumor cells from an individual focus in gastrinomas have been reported to be genetically clonal in origin (31, 32).

Fig. 1D shows an example of MSI. This was manifested by extra peaks in the tumor DNA PCR product that were not present in the normal DNA product. It was seen in 8% of the markers from patient 7, who had a large and unusually aggressive tumor, and was not seen in any of the other patients. In total, just over 6000 PCR reactions were performed using about 400 different markers. Allele homozygosity resulting in a noninformative result was found in 20% of the reactions, and this value is in

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Course*</th>
<th>Size*</th>
<th>Tumor location*</th>
<th>MEN1 Defect</th>
<th>Lymph node metastases</th>
<th>Hepatic metastases</th>
<th>Defect location*</th>
<th>Aneuploidy*</th>
<th>MSI</th>
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<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Small</td>
<td>Liver</td>
<td>--</td>
<td>U*</td>
<td>+</td>
<td>11q</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>Large</td>
<td>Lymph node</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>1p, 1q</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>Large</td>
<td>Lymph node</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>1q, 7p</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>Small</td>
<td>Duodenum</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>Small</td>
<td>Duodenum</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>11q</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>Large</td>
<td>Lymph node</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>9</td>
<td>--</td>
<td></td>
</tr>
<tr>
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<td>Pancreas</td>
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<td>13</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>I</td>
<td>Small</td>
<td>Lymph node</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>

* I, indolent; A, aggressive.

* Small, a tumor < 3 cm in diameter; large, a tumor > 3 cm in diameter.

* Source of tumor DNA used in the study.

* U, unknown.

* Chromosome arm showing defect.

* Total number of chromosomes showing AI at every informative marker.

The 16 DNA samples from the eight patients (blood and tumor DNA from each patient) were individually added to each of the 414 pairs of primers described above using a Biomek 2000 robotic workstation (Beckman Instruments, Inc., Fullerton, CA). Each PCR reaction was conducted in a total volume of 15 µl containing 100 ng of DNA, 0.27 µM each primer, 250 µM each deoxynucleotide triphosphate, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, and 5 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer). Reactions were performed in a Perkin-Elmer GeneAmp PCR system 9600 thermal cycler as follows: (a) denaturation at 95°C for 12 min; (b) 10 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 60 s; (c) 20 cycles of denaturation at 89°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 60s; and (d) extension at 72°C for 90 min.

Samples were run on an ABI 377 sequencer (Perkin-Elmer), and because the primers contained one of three different fluorescent labels, and the markers had different size ranges, about eight reactions could be pooled and run in each lane of the gels. Peak heights and areas were quantified, and the data were processed using the Genescan and Genotyper software programs provided with the sequencer.

CGH. After the full complement of PCR reactions described above were performed, the nature of the defects detected was confirmed by CGH. Because the amount of tumor DNA available at this point was limited, analysis was not attempted on patients 7 and 8. In addition, the tumor DNA was not used directly for CGH but was amplified using degenerate oligonucleotide-primed PCR (27, 28). Two steps were used to amplify the DNA, with the first (preamplification) step done as described previously (27). The second step was conducted by adding each of the preamplified DNA samples to 50 µl of a solution that contained 1.5 µM UN1 primer (27), 200 µM each deoxynucleotide triphosphate, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, and 5 units of AmpliTaq DNA polymerase. Reactions were performed in a PTC-200 thermal cycler (MJ Research, Inc., Waltham, MA) using 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 2 min. A DNA sample from the MCF-7 breast cancer cell line containing well-characterized alterations was used as a positive control, and DNA samples from male and female normal lymphocytes were used as the source of reference DNA.

The hybridization protocol was performed as described previously (29). Briefly, the DNA samples were labeled by nick translation using FITC-12-dUTP (DuPont, Boston, MA), and normal reference DNA was labeled using Texas red-6-dUTP (DuPont). Five hundred ng of labeled sample DNA were added to 300 ng of labeled sex-matched reference DNA and 10 µg of Cot-1 DNA (Boehringer Mannheim GmbH, Mannheim, Germany). Hybridization to normal metaphase preparations was done for 48 h, and, after washing, the slides were mounted in an antifade solution containing 4',6-diamidine-2-phenylindole counterstain. The ratios of green:red fluorescence intensities (tumor:normal) were analyzed along each autosome and the X chromosome using a digital image analysis system made up of a BX50 fluorescence microscope (Olympus, Tokyo, Japan) and an Image-Point charge-coupled device camera (Photometrics Ltd, Tucson, AZ) equipped with IPLab software (Scanalytics, Inc., Fairfax, VA). Images were further analyzed using the Quips CGH software package (Vysis, Inc., Downers Grove, IL).
has a value. That is, a normal result as seen in Fig. 1. A white portion shows the magnitude of AI for the eight tumors. The remaining columns are labeled NP, and insufficient or ambiguous products (MI, MSI noninformative because of homozygosity (H), that is almost completely white. Results that were an AI value near unity and is represented by a bar—d. Large defects involving multiple adjacent markers or even entire chromosomes were found in 10% of the reactions, and these areas are listed in Table 1. Of the remaining 52% of the reactions, the mean of the ratio (ab/l)/(cd) was 1.04 with a SD of 0.18, and this gives an estimate of the precision of the method when assaying genomic regions free of at least large rearrangements.

Chromosome 11 Defects. Thirteen markers from chromosome 11 were used, and the results for all eight patients are displayed in Fig. 2. In this figure, the markers are arranged sequentially from the tip of the short arm of chromosome 11 (top) to the tip of the long arm (bottom). The result for each marker for each patient is represented by a bar, and the amount of white in each bar is proportional to the ratio (ab/l)/(cd).

A typical example of LOH would have a ratio of 0.2, and in this case, 20% of the bar would be white, and 80% would be black. For purposes of ease in using the data processing programs, the lower molecular weight alleles in blood or tumor DNA were always labeled a or c, respectively, following the convention shown in Fig. 1A. A result of this convention is that half the abnormal ratios were greater than unity, and half were less than unity, depending on whether the lower or higher molecular weight allele in the tumor was affected. A ratio of 0.2, however, would have the same molecular significance as a ratio of 5, and would be displayed identically in Figs. 2–4 by plotting the reciprocal of the ratios that were greater than unity.

In patient 7, every informative marker on chromosome 11 showed LOH. Whereas other more complicated explanations are possible, the simplest explanation is that the patient’s tumor had monosomy of this chromosome. In contrast, there is no evidence of an abnormality in patients 2, 3, 4, 6, and 8 on chromosome 11. One caveat is that sampling of the chromosome was done at intervals of 10 cM on average (about 10 megabases), so that the technique probably would have failed to detect smaller rearrangements. In addition, in 48% of the reactions done in this study, the product from a given marker was noninformative because of homozygosity (H in Figs. 2–4), MSI (MI), or other reasons including insufficient yield from the PCR reaction (NP). Whereas this is not a significant issue when there are multiple informative markers showing AI, it is an issue when evaluating areas such as those in patients 1 and 5 on chromosome 11. In these two patients, there are markers showing LOH on 11q, but an especially interesting area is the region of the MEN1 gene at the chromosome band 11q13 (70–80 cM). Whereas markers on the telomeric side of the MEN1 region show LOH, the markers on the centromeric side are noninformative or normal. Large deletions of this region are common in gastrinomas (6, 9, 10, 31, 32), and when one evaluates for LOH in the immediate vicinity of the MEN1 gene, deletions are almost universally found (7). Thus, as a validation of the method, finer mapping around the MEN1 gene was performed in patients 1 and 5 using four additional markers, and these results confirmed LOH of the MEN1 gene region (data not shown).

**Chromosome 11**

<table>
<thead>
<tr>
<th>Marker</th>
<th>cM</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<td>0</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>M1</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>D15S1956</td>
<td>6</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>M1</td>
<td>NP</td>
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</tr>
<tr>
<td>D15S199</td>
<td>14</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>H</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>D15S1991</td>
<td>26</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>H</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>A1AT/C1</td>
<td>34</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>H</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>D15S237</td>
<td>72</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>H</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>D15S202</td>
<td>82</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>H</td>
<td>NP</td>
<td>NP</td>
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<tr>
<td>D16S1959</td>
<td>110</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>NP</td>
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<tr>
<td>D15S1344</td>
<td>118</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<td>NP</td>
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<tr>
<td>D16S526</td>
<td>137</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>

**Fig. 1.** Examples of tracings from individual microsatellite markers. A, example of a normal trace from normal blood DNA (N) and tumor DNA (T). Peaks a–d were labeled and analyzed as described in the text. The results shown are for patient 8 at locus D2S1776. B, example of LOH from patient 7 at locus D2S1776. C, example of AI that is not as extreme as that typically seen in LOH. The tracing is from patient 8 at locus D8S1130. D, example of MSI seen in patient 8 at locus D2S454.

**Fig. 2.** Compilation of results for chromosome 11. The marker name is in the first column, and its genetic map position (in cM) is in the second column. Markers are listed in order from the tip of the short arm of the chromosome (top) to the tip of the long arm (bottom). The remaining columns show the magnitude of AI for the eight tumors. The white portion of each bar is proportional to the AI value. That is, a normal result as seen in Fig. 1A has an AI value near unity and is represented by a bar that is almost completely white. Results that were noninformative because of homozygosity (H), MSI (MI), and insufficient or ambiguous products (NP) are labeled.
Chromosome 5 Defects and Aneuploidy. The results from the chromosome 5 markers for all eight patients are shown in Fig. 3. Patients 2, 6, and 7 showed AI at every informative marker throughout the chromosome. Patient 7 had marked AI consistent with LOH, identical to the results seen in this patient on chromosome 11. Whereas the tracings in patient 7 showed AI of the magnitude depicted in the example shown in Fig. 1B, patients 2 and 6 showed a lesser extent of AI similar to the result shown in Fig. 1C. Thus, it is highly likely that the molecular defect involving chromosome 5 in patients 2 and 6 is different than the defect found in patient 7.

Chromosome 1

Whereas the tracings in patient 7 showed AI of the magnitude depicted in the example shown in Fig. 1B, patients 2 and 6 showed a lesser extent of AI similar to the result shown in Fig. 1C. Thus, it is highly likely that the molecular defect involving chromosome 5 in patients 2 and 6 is different than the defect found in patient 7.
discussed above, an attractive and simple explanation is that patients 2 and 6 were trisomic for chromosome 5, and patient 7 was monosomic. Some additional data to support this explanation were obtained in the CGH experiments discussed later.

AI involving every informative marker of an entire tumor chromosome was especially common in patients 6 and 7. Specifically, patient 6 had AI of nine different chromosomes, and besides the results shown in Fig. 3 for chromosome 5, this patient’s tumor also showed AI throughout chromosomes 6, 9, 13, 15, 16, 17, 18, and X. Patient 7, who had a particularly aggressive tumor, had AI throughout 13 different chromosomes. The results for chromosomes 11, 5, and 1 are shown in Figs. 2–4, and similar results were also seen for chromosomes 2, 3, 6, 8, 9, 10, 15, 16, 18, and 22. As shown in Fig. 3, patient 2 had AI throughout chromosome 5, but this tumor had no other defects that involved an entire chromosome. Similarly, patient 3 had AI throughout the X chromosome alone. As summarized in Table 1, these four patients all had large or aggressive tumors, and such defects were not seen in the four patients with small tumors.

Strictly speaking, the term aneuploidy refers to an abnormal number of chromosomes, but this is usually in the context of abnormal DNA content detected by more direct techniques such as karyotype analysis of metaphase cells or flow cytometry. The PCR-based allelotyping studies used here can detect abnormal DNA content for each chromosome, although the experiments do not directly show whether the content is above or below 2n (n, haploid genome equivalent of DNA). In addition, the results do not directly prove that the abnormality is continuous or corresponds to a cytogenetically distinct structure. For example, LOH at every informative marker on a chromosome could be due to monosomy but does not rule out the possibility that a more complicated deletion exists. Such a scenario might involve deletion of the short arm of one chromosome coexisting with deletion of the long arm of its homologue. Whereas this scenario would probably be functionally identical to monosomy of the chromosome, it would not meet the cytogenetic criteria for monosomy or aneuploidy.

**Chromosome 1 Defects.** The largest and most complicated set of defects were seen on chromosome 1. Three patients had abnormalities seen in this chromosome after the initial screening with 24 markers was done, and these defects were further investigated by supplementing the screen with an additional 23 markers distributed throughout the chromosome. The results are shown in Fig. 4. Patient 7 had LOH at every informative marker, but the results for patients 2 and 3 were more complicated. Specifically, patient 2 had normal results at the end of 1p from 0–62 cM, marked AI indicative of LOH on the rest of 1p from 70–156 cM, and milder AI on 1q from 167 cM to the end of 1q. Patient 3 had normal results on 1p but had two areas of LOH on 1q at 167–221 cM and from 251 cM to the end of 1q. The AI patterns in both patients show a discontinuity in the pericentric region between 156 and 167 cM, and this area warrants further analysis as a possible hot spot of rearrangement.

**CGH Analysis.** To confirm and extend the allelotyping results, CGH was performed on patients 1–6 (insufficient DNA remained from patients 7 and 8). The CGH technique has the potential to supplement AI measurements by indicating whether any large DNA region is present above or below 2n and by quantifying the copy number. It is also a very sensitive technique for detecting high-copy number amplifications of large regions; however, the technique is not consistently accurate in detecting copy number changes of 1n that are a result of a deletion, a duplication, monosomy, or trisomy (27, 29).

In contrast to what is usually seen in solid tumors, the tumors in patients 1–6 had relatively few abnormal areas. In particular, no areas of high-level amplification were detected. The abnormal areas that were detected by both CGH and the AI method are shown in Fig. 5. The chromosome 1 defects in patients 2 and 3 were detected using CGH, although the magnitude and position of the defects were probably not determined as precisely as they were with the AI results discussed above. Nevertheless, the CGH results do support the interpretation that patient 2 has LOH resulting from a deletion on 1p and has a duplication of 1q, whereas patient 3 has at least two areas of LOH resulting from deletions on 1q. Also shown are the results from chromosome 5 in patient 2, and the CGH results, when combined with the AI results shown in Fig. 3, support an interpretation of trisomy for this chromosome.

**DISCUSSION**

Relatively little is known about the molecular biology of gastrinomas. The largest factor contributing to the deficiency is the rarity of this type of tumor, and this is reflected in the small sample size of the present study, as well as in previous studies. Another factor is the availability of sensitive tumor-imaging methodologies for localization. Consequently, many surgically resected specimens do not yield sufficient material for analysis. In this study, we have compensated by using sensitive PCR-based techniques and performing an in-depth analysis at 400 different loci as well as including the whole-genome approach of CGH.

Whereas this study is somewhat unusual in the large number of loci examined, results using the same basic techniques are available for numerous other types of tumors. Overall, the results in gastrinomas show that large deletions or amplifications are relatively rare and that the genomes of these tumors are generally much more stable than those of other types of malignancies. This is consistent with the clinical observation that sporadic gastrinomas generally grow slowly and have a 10-year survival rate of 94% (1).

Despite this background of genetic stability, several striking genetic defects were detected. Multiple large defects were found on chromosome 1 in two patients, and both patients had a discontinuity in the pattern of AI in the same pericentromeric region of the chromosome. Whereas this interval is several megabases in size and was not mapped with better resolution, it is possible that this area is a hot spot for chromosome breakage. Interestingly, in a recent compilation of rearrangements common in human neoplasia, the pericentromeric region of chromosome 1 was especially well represented (33). The results in these two patients, both of whom had hepatic metastases, also confirm and extend a previous observation of chromosome 1 defects in gastrinomas (19). In that study, 4 of 18 gastrinomas showed LOH on chromosome 1, and this group included all three of the
patients who had hepatic metastases. However, the study had limited information on the location of breakpoint regions because only 12 markers were used, and two of the four abnormal gastrinomas had LOH at every informative marker. An additional study (20) found LOH on chromosome 1 in pancreatic tumors, but only two endocrine tumors of unspecified phenotype and two markers on chromosome 1 were included.

The rationale for performing genome mapping studies is that tumor suppressor genes or oncogenes might be present in commonly rearranged regions or at breakpoints. In this regard, the pericentric region of chromosome 1 is known to contain the N-ras and β subunit of nerve growth factor genes. In colon cancers, the mutations of the K-ras gene have been correlated with increased gastrin expression (34). Whereas the N-ras, K-ras, and H-ras genes have been sequenced in gastrinomas, no mutations have been found (17, 35, 36). It is possible that genetic alterations other than point mutations are important or that mutations are found in only a subset of tumors. Besides the MEN1 gene, no other obvious genes implicated in gastrin expression or regulation are known to be present in the abnormal regions detected in this study. However, the number of components in these pathways is rapidly expanding, and the MEN1 gene product has recently been shown to bind to JunD (15) and to revert the morphology of RAS-transformed NIH3T3 cells (37).

Although we do not have cytogenetic data, presumptive aneuploidy was detected in several chromosomes that had AI at every informative marker. This result was seen only in the four tumors that were large or aggressive and was not seen in the other four tumors that were indolent and small. This confirms a previous flow cytometry study from this institution (using a different set of tumor specimens) in which aneuploidy (of the multiple stem line type) was found only in aggressive metastatic tumors (23).

MSI, presumably due to a defect in a mismatch repair pathway, was observed in only one patient who had a large and unusually aggressive tumor. Whereas MSI has been described in multiple different solid tumors including carcinoid tumors of the lung (38) and the gastrointestinal tract (39), it has not been reported previously in gastrinomas (40). Given the large number of microsatellite analyses reported previously, especially in the MEN1 gene search literature, MSI should now be considered a possible but very rare event in gastrinomas. In this regard, gastrinomas are almost identical to stromal tumors of the gastrointestinal tract. In a recent report (41), MSI was found in only 1 of 30 stromal tumor specimens, whereas LOH was a common event. In contrast, leiomyomas have an excellent clinical prognosis, like gastrointestinal tract. In a recent report (41), MSI was found in only 40% (44).

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MSI, presumably due to a defect in a mismatch repair pathway, was observed in only one patient who had a large and unusually aggressive tumor. Whereas MSI has been described in multiple different solid tumors including carcinoid tumors of the lung (38) and the gastrointestinal tract (39), it has not been reported previously in gastrinomas (40). Given the large number of microsatellite analyses reported previously, especially in the MEN1 gene search literature, MSI should now be considered a possible but very rare event in gastrinomas. In this regard, gastrinomas are almost identical to stromal tumors of the gastrointestinal tract. In a recent report (41), MSI was found in only 1 of 30 stromal tumor specimens, whereas LOH was a common event. In contrast, leiomyomas have an excellent clinical prognosis, like gastrointestinal tract. In a recent report (41), MSI was found in only 40% (44).

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GENETIC ALTERATIONS IN GASTRINOMAS


Survey of Genetic Alterations in Gastrinomas
Fang Yu, Robert T. Jensen, Irina A. Lubensky, et al.

*Cancer Res* 2000;60:5536-5542.

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