Allelic Loss on Chromosome 11 in Hereditary and Sporadic Tumors Related to Familial Multiple Endocrine Neoplasia Type 1

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ABSTRACT

Familial multiple endocrine neoplasia type 1 (FMEN1) is an autosomal dominant disorder characterized by tumors of the parathyroid glands, pancreatic islets, and anterior pituitary. The gene for this disease maps to chromosome 11q12–11q13, and allelic loss in this region has been shown in both sporadic and FMEN1-related parathyroid tumors, FMEN1-related pancreatic islet tumors, and rarely in sporadic anterior pituitary tumors. We tested for allelic loss at 7 loci on chromosome 11 in 17 tumors outside the parathyroid. We found loss of heterozygosity in 2 of 2 FMEN1-related benign pancreatic islet tumors but in none of 8 informative sporadic islet tumors (P = 0.02) including 5 malignant gastrinomas. Of 3 islet tumors from patients who had some but not all features of FMEN1, one showed allelic loss for 5 of 5 informative restriction fragment length polymorphisms, and the other 2 retained heterozygosity for all informative markers. A bronchial carcinoid from an FMEN1 patient and 3 sporadic anterior pituitary tumors showed no allelic loss. These data provide new evidence that many sporadic pancreatic islet neoplasms, even when malignant, do not develop through homozygous inactivation of the MEN1 gene.

INTRODUCTION

The study of hereditary cancer predisposition syndromes has helped to elucidate the molecular basis of carcinogenesis. In 1971 Knudson (1) proposed that tumorigenesis requires two genetic events or “hits” in a tumor clone precursor cell based on clinical observations of the number and distribution of malignancies in Rb. Mapping of the Rb gene to chromosome 13 (2) made possible a direct experimental test of the two hit theory. By comparing the chromosome 13 RFLP patterns in tumor DNA with constitutional DNA, Cavenee et al. (3) provided evidence that tumors arise when both copies of the Rb gene in a retinal cell become defective through either two somatic events (sporadic Rb) or a single somatic event affecting the normal homologue in an individual with an hereditary first hit. Analogous loss of the normal homologue of a growth suppressor gene has been observed in acoustic neuroma (4), colon carcinoma (5), Wilms’ tumor, and other tumors (6). Presumably the action of these genes is recessive at the cellular level.

FMEN1 is an autosomal dominant disorder characterized by overgrowth and hyperfunction of the parathyroids, anterior pituitary, and pancreatic islet cells (7). Tumors seen less frequently include carcinoids and thyroid adenomas. Although the prevalence is unknown, approximately 1–3% of primary hyperparathyroidism and 25% of gastrinomas have been attributed to this disorder (7–9). The diagnosis of FMEN1 is usually made in the third to fourth decade, but the first signs, often referable to the parathyroid, may begin prior to age 20. FMEN1 is clinically similar to hereditary Rb in that the associated tumors are multifocal and have a relatively early age of onset compared to that in sporadic cases.

Larsson et al. (10) reported linkage of the MEN1 gene to the muscle phosphorylase locus on chromosome 11q12–11q13 and showed loss of one entire copy of chromosome 11 in malignant insulinomas from two affected individuals. Apparently some or all tumors in this disorder arise when a susceptible cell develops a homozygous defect in the MEN1 gene. By analogy to Rb it seems very likely that the MEN1 gene is a coding sequence and its product is a trans-acting growth suppressor.

Additional evidence has accumulated for the two hit mechanism of neoplasia in FMEN1. The majority of parathyroid tumors from FMEN1 patients (11–13) and 25% of sporadic parathyroid adenomas (11, 13) show allelic loss for chromosome 11 markers. Chromosome 11 allelic loss was not observed in one FMEN1-related pituitary tumor analyzed and was found only in 2 of 26 sporadic pituitary tumors (13).

The purpose of the current study was to determine whether allelic loss from chromosome 11 is a general phenomenon in neoplasms outside the parathyroids in patients with FMEN1 and to determine whether or not sporadic tumors of the types seen in FMEN1 develop with a similar mechanism.

MATERIALS AND METHODS

Clinical Data. Eleven pancreatic islet tumors, 3 anterior pituitary tumors, and 1 bronchial carcinoid were obtained from patients who underwent surgery at the NIH. Two pancreatic islet tumors were from patients who underwent surgery elsewhere. Tumor type was assigned according to the associated clinical endocrinopathy. Histology reports for all tumors were reviewed. Tumors were classified as malignant if local or distant metastases were documented. Efforts were made to obtain a complete family history relevant to FMEN1 from all patients. Patients were diagnosed as definitely affected with FMEN1 if, in addition to a typical endocrine neoplasm, they had at least two relatives with FMEN1-related endocrinopathies and at least one with multiple organs affected. In cases classified as sporadic, there was no family history suggesting FMEN1, and the patient expressed only one FMEN1-related endocrine abnormality. Possible FMEN1 was diagnosed in patients with multiple endocrine tumors typical of FMEN1 but without a family history diagnostic of FMEN1 or in those with a

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3 The abbreviations used are: Rb, retinoblastoma; RFLP, restriction fragment length polymorphism; FMEN1, familial multiple endocrine neoplasia type 1; VIP, vasoactive intestinal peptide.

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family history suggestive but not diagnostic of FMEN1 and endocrine neoplasia affecting only one organ.

Laboratory Techniques. All tumors were dissected from surrounding normal tissue and stored at −20°C prior to use. Tissues were pulverized in dry ice, and DNA was prepared using the guanidine hydrochloride technique (14). Peripheral blood leukocyte DNA was extracted from either fresh or frozen cells using the same technique. DNA (5 µg) was digested to completion according to the manufacturer’s instructions and size fractionated by agarose gel electrophoresis. Transfer to nylon membranes (Hybond N; Amersham) was performed according to manufacturer’s instructions.

The probes used in this study (15) [pINS-310 from the insulin locus at 11p15.5; pTHH26 (D11S149), probe PGA101 (PG43), probe pMCMPl (PYGM), probe pHBI59 (D11S146), and probe SS6 (INT2), from 11pl3; and probe L7 (D11S29) from chromosome 11q23] were labeled to a specific activity of 10^6 cpm/µg according to manufacturer’s instructions (Boehringer Mannheim Random Primed DNA Labelling Kit). Prehybridization and washing conditions were essentially those described by the manufacturer (Amersham) for high stringency. Filters were autoradiographed (Kodak XAR5 film) at −70°C for 1 to 7 days.

Allelic Losses from Chromosome 11

Pancreatic Islet Neoplasms. Both islet tumors from FMEN1 patients were informative for at least 2 RFLPs with at least 1 in the 11q13 region, and loss of heterozygosity was seen for all informative loci (Table 1; Fig. 1). There was no evidence that either of the tumors from an FMEN1 patient retained heterozygosity at any portion of chromosome 11. However, in one case only 2 RFLPs, both close to the MEN1 gene, were informative.

All 8 sporadic islet tumors were informative for at least 1 chromosome 11 RFLP: 5 of the 8 for 1 or more in 11q13; and 3 only for markers on the distal p or q arms. No loss of heterozygosity was observed in any of these tumors, a significant difference when compared with the tumors from FMEN1 patients (P = 0.02, Fischer’s exact test).

Each of the three islet tumors from patients who were difficult to classify with regard to FMEN1 was informative for markers in 11q12–11q13. The islet tumor from a patient with associated primary hyperparathyroidism but no known family history of FMEN1 showed allelic loss for 5 of 5 RFLPs. Neither of the other two islet tumors from possible FMEN1 patients showed any allelic loss.

RESULTS

Diagnostic Classification of Patients

Eight pancreatic islet tumors and 3 pituitary tumors were classified as sporadic (Table 1). Two pancreatic islet tumors and one bronchial carcinoid were classified as from patients with FMEN1. The remaining three pancreatic islet tumors were classified as from patients with possible FMEN1 because two patients had associated endocrine abnormalities but no family history of FMEN1, and one patient had a family history suggestive of gastrinoma but no other features of FMEN1.

| Table 1 Clinical findings in patients from whom tumors were obtained
<table>
<thead>
<tr>
<th>Tumor code</th>
<th>Tumor type</th>
<th>Malignant (M) or benign (B)</th>
<th>Associated neoplasms and endocrine abnormalities</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>08</td>
<td>Insulinoma</td>
<td>B</td>
<td>Acromegaly (7GHRH* from islet tumor)</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Nonsecreting islet tumor</td>
<td>M</td>
<td>S/P parathyroidectomy, bladder cancer, Hodgkin’s disease</td>
<td>Negative</td>
</tr>
<tr>
<td>21</td>
<td>Gastrinoma</td>
<td>M</td>
<td>None</td>
<td>Father had recurrent peptic ulcers</td>
</tr>
<tr>
<td>15</td>
<td>Bronchial carcinoid</td>
<td>B</td>
<td>S/P parathyroidectomy, Zollinger-Ellison syndrome</td>
<td>5 relatives with FMEN1</td>
</tr>
<tr>
<td>17</td>
<td>VIPoma</td>
<td>B</td>
<td>Hyperparathyroidism, Zollinger-Ellison syndrome, prolactinoma</td>
<td>2 relatives with FMEN1</td>
</tr>
<tr>
<td>18</td>
<td>Insulinoma</td>
<td>B</td>
<td>Hyperparathyroidism, Zollinger-Ellison syndrome, prolactinoma</td>
<td>6 relatives with FMEN1</td>
</tr>
</tbody>
</table>

* Tumor type and malignancy were assigned according to histology, associated endocrinopathy, and presence of metastases. Sporadic, a single endocrine disorder and no family history of FMEN1; possible FMEN1, multiple endocrine tumors typical of FMEN1 without a family history of FMEN1 or a single endocrine tumor and a family history suggestive of FMEN1; definite FMEN1, a typical endocrine abnormality and at least two relatives with FMEN1.

* GHRH, growth hormone-releasing hormone; s/p, status post.
Allelic loss in MEN1 tumors

Other Tumors. All 3 sporadic pituitary tumors studied were informative for at least 2 markers in the FMEN1 region and retained heterozygosity. The 1 bronchial carcinoid from an FMEN1 patient was informative for 2 RFLPs in 11q12-q13 and failed to show any loss of heterozygosity. PYGM, the marker most closely linked to the MEN1 gene, and all distal 11q markers were uninformative; therefore allelic loss including the FMEN1 locus and distal regions could not be excluded.

Discussion

Hereditary disorders predisposing to neoplasia in humans fall into two major classes. DNA repair defects (16), such as xeroderma pigmentosum, lead to extreme sensitivity to carcinogenic agents because affected individuals cannot repair DNA damage caused by such agents. These disorders are uniformly autosomal recessive. Another type of defect, which is responsible for a variety of autosomal dominant disorders, involves mutations inactivating “antioncogenes” (17). In disorders of this latter type individuals inherit a mutation in one homologue of the responsible gene, and a susceptible cell becomes neoplastic when the other homologue is inactivated through mutation, deletion, or mitotic recombination. Frequently, sporadic tumors of the same type seen in these antioncogene diseases develop with accumulation of two somatic events in one cell, leading to homozygous inactivation of the gene responsible for the hereditary form. In either the hereditary or sporadic form comparison of constitutional with tumor DNA often shows allelic loss for the disease gene itself and frequently for surrounding polymorphisms.

In our study two benign islet tumors from patients who definitely had FMEN1 showed allelic loss in the region of the MEN1 gene. These data extend Larson’s finding of chromosome 11 allelic loss in 2 malignant insulinomas in FMEN1 (10) to benign islet tumors (insulinoma and VIPoma) in FMEN1. None of the 11 sporadic islet tumors (5 benign and 6 malignant) tested by us showed allelic loss from chromosome 11. The difference between the proportion of FMEN1-associated and sporadic islet tumors with allelic loss was significant. It is possible that sporadic islet tumors have homozygous small interstitial deletions or point mutations affecting the MEN1 gene not detected by this study. In addition allelic loss could have been obscured in sporadic tumors due to admixture of normal cells; but tumors showing allelic loss and those with no loss of heterozygosity were handled similarly and were grossly free of surrounding normal tissue. It is most likely that sporadic tumors do not arise through homozygous inactivation of the MEN1 gene. None of 7 gastrinomas (6 sporadic and 1 possible FMEN1-associated) showed allelic loss for chromosome 11 RFLPs although each was malignant; and it is unclear if this is a feature specific to sporadic gastrinomas, most gastrinomas, or most sporadic islet tumors. One other important difference between sporadic and FMEN1-associated gastrinoma has been reported; sporadic gastrinoma generally arises in the pancreas while that in FMEN1 often arises in the duodenal mucosa (18).

These data contrast with the findings in retinoblastoma where both hereditary and sporadic tumors show homozygous inactivation of the RB gene. We have not identified a genetic locus affected during tumor development in sporadic gastrinoma. The concept of separate areas of allelic loss in sporadic and hereditary forms is not without precedent and has been demonstrated for colon adenomas and colon cancer (19). However, in contrast to our results, tumors from patients with familial adenomatous polyposis failed to show allelic loss in the region of the familial adenomatous polyposis gene, but loss of this region was seen in one-third of sporadic tumors (20).

Our analysis of sporadic pituitary tumors failed to show any loss of heterozygosity for chromosome 11 RFLPs and suggests that these tumors do not arise through homozygous inactivation of the MEN1 gene. The mechanism of tumorigenesis in FMEN1-related pituitary tumors was not addressed by our study. Approximately 25% of monocular growth hormone-secreting pituitary tumors seem to develop through a point mutation that inhibits GTPase activity of the α chain of the stimulatory G protein (20).

The failure to detect allelic loss in the one bronchial carcinoid from a patient with FMEN1 was somewhat surprising given the high frequency of chromosome 11 allelic loss in other types of endocrine tumors from FMEN1 patients. However, no markers extremely close to the MEN1 gene were informative, and informative markers distal to the gene were not identified. Therefore, it is possible that either a small interstitial deletion or a deletion including the MEN1 gene and more distal parts of chromosome 11 was not detected. Analysis of this tumor with additional RFLPs and typing of bronchial carcinoids from other FMEN1 patients as well as sporadic bronchial carcinoids will help determine the mechanism of tumorigenesis in this form of neoplasia.

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References

ALLELIC LOSS IN MENI TUMORS


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