Allelic Deletions on Chromosome 11q13 in Multiple Tumors from Individual MEN1 Patients

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ABSTRACT

Familial multiple endocrine neoplasia type 1 is an autosomal dominant hereditary disorder characterized by multiple parathyroid, pancreatic, duodenal, and pituitary tumors. The parathyroid tumors may arise as diffuse areas of hyperplasia, whereas the pancreatic and duodenal tumors usually form as discrete nodules. Except for a single report, tumor loss of heterozygosity (LOH) mapping of the putative MEN1 suppressor gene on chromosome 11q13 in the past has been restricted by analysis of a single tumor from individual patients and somatic cellular contamination. For this reason, it has not been possible to analyze the clonality of the emerging MEN1 neoplasms. Furthermore, it has been previously unknown whether the LOH pattern varies between individual MEN1 tumors in a given patient or among tumors of different histological origins within unrelated patients. To address these previous limitations, the present study introduces a refinement in microdissection in which endothelial cells are stained and selectively excluded. Tissue microdissection was applied to study LOH patterns on chromosome 11q13 using 8 polymorphic DNA markers in 44 different MEN1 tumors from parathyroid, pancreatic, and duodenal in nine unrelated patients. In addition, X-chromosome inactivation clonal analysis was applied to 16 individual microdissected regions from seven parathyroid glands in three female patients. The LOH rates of parathyroid lesions (100%) and endocrine tumors of the pancreas (83%) were strikingly different from the LOH rate of gastrinomas (21%), suggesting that the mechanism that drives LOH may be influenced by the tissue context. Moreover, combined LOH and X-chromosome inactivation scoring of the same microdissected region revealed that parathyroid MEN1 neoplasms can consist of more than one clone. In this study, the centromeric boundary of the putative MEN1 gene was PYGM. Analysis of differential LOH patterns in multiple microdissected tumors in the same patient constitutes a novel approach to suppressor gene mapping.

INTRODUCTION

FMEN1 is an autosomal dominant hereditary disorder characterized by tumors of multiple parathyroid glands (90–97%), endocrine pancreas (30–82%), and duodenum (25–60%) and adenomas of anterior pituitary gland (60%; Refs. 1–3). In contrast to enlarged parathyroid glands with histologically confluent diffuse and nodular hypercellular areas, the tumors of pancreas and duodenum are small, multifocal discrete nodules. The putative MEN1 tumor suppressor gene has been linked to chromosome 11q13 (4, 5). Previously, LOH in the 11q13 region has been detected in 42 of 67 (63%) MEN1-related parathyroid lesions (6–12) and in 11 of 12 (92%) pancreatic endocrine tumors (nongastrinomas; Refs. 4, 8, 9, 13–15). However, MEN1 studies to date, with one exception (11), have been restricted to LOH analysis of single parathyroid glands or single pancreatic endocrine neoplasms from individual members of MEN1 kindreds.

The lesions in FMEN1 tend to be multiple in a given patient and affect several organs with increasing patient age (16). In the individual patient, the genotype of each neoplasm is assumed to consist of an inherited germ line mutation of the MEN1 gene and loss of function of the wild-type allele through chromosomal deletion or point mutations (4, 17). It has been previously unknown whether the LOH pattern of the wild-type allele varies between individual MEN1 tumors in a given patient or among tumors of different histological origins within unrelated patients.

As a novel approach to tumor suppressor gene mapping we used tissue microdissection to study 44 histologically different tumors from parathyroid glands, pancreas, and duodenum in nine unrelated FMEN1 patients to investigate patterns of LOH on 11q13. We introduced a new refinement in the tumor microdissection technique to eliminate endothelial cells, thus reducing contamination from these cells in tumor tissue. The LOH patterns in parathyroid lesions and nonparathyroid MEN1 tumors were compared in the individual patients and among unrelated patients with FMEN1. In addition, X-chromosome inactivation analysis of 16 individual regions in seven parathyroid glands from three female patients was performed to investigate clonality of MEN1-associated parathyroid lesions, and the results were correlated with the LOH data.

MATERIALS AND METHODS

Tissue Samples. Forty-four endocrine lesions from nine unrelated patients with familial MEN1 were studied. Patients were diagnosed as affected with FMEN1 if, in addition to two typical endocrine neoplasms, they had at least two first-degree relatives with MEN1-related endocrinopathies. Normal samples and 80 tumor samples, which were formalin-fixed, paraffin-embedded (from seven patients; patients 1, 3–6, 8, and 9), and frozen (from three patients; patients 2, 7, and 8), were obtained from the National Cancer Institute Laboratory of Pathology file. The endocrine lesions included 19 enlarged parathyroid glands in seven patients and 10 gastrinomas of the duodenum, 4 endocrine tumors of the pancreas (2 insulinomas, 2 nonfunctional tumors), 1 insulinoma, and 10 gastrinoma metastases in five patients (Tables 1–3). In seven patients, two or more parathyroid glands were found to be abnormal by size (range, 0.8–3.6 cm), and intraoperative and postoperative histopathological evaluation confirmed hypercellularity of each gland. The term “hyperplasia” was used when two or more parathyroid glands in one patient showed prominent hypercellularity. Nineteen hypercellular parathyroid glands were divided into 55 separate microscopic regions. These regions were composed of either distinct nodules or solid sheets of chief or oxyphil cells (Fig. 1). Each region in parathyroid gland was circled on the glass slide, and assigned a sequential number (Table 2). In addition, parathyroid sections were immunostained for endothelial cells with an anti-CD34 antibody (QBEND 10, AMAC, Inc.; Ref. 18), and precise microdissection of parathyroid cells was performed to avoid endothelial contamination (Fig. 1). Histological sections from endocrine tumors of the duodenum (size, 0.5–1.5 cm) and pancreas (size, 0.5–3 cm) and from metastases (size, 2–14 cm) were reviewed. Each tumor was assigned a consecutive number and immunostained with antibodies to chromogranin (Boehringer Mannheim), synaptophysin (Zymed, San Francisco, CA), gastrin (DAKO, Carpinteria, CA), insulin (BioGenex, San Ramon, CA), and pancreatic polypeptide (DAKO, Carpinteria, CA), and areas of interest were selected for microdissection. Anti-CD34 immunostain in these tumors did not show a

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2The abbreviations used are: FMEN1, familial multiple endocrine neoplasia type 1; HUMARA, human androgen receptor; LOH, loss of heterozygosity.

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significant endothelial component. Normal tissue samples for each patient included lymph nodes, thymic tissue, or exocrine pancreas removed at the time of surgery.

**Microdissection.** Tumor and normal cells were selected from the 5-μm-thick H&E- and CD34-stained slides and microdissected using a modified Pasteur pipette or 30-gauge needle under light microscopic visualization (19, 20). Precise microdissection of parathyroid cells was performed between the vessels to minimize endothelial contamination (Fig. 1). Each tumor region was microdissected from 4 or 5 consecutive histological slides in the case of parathyroid glands and from 2 or 3 consecutive histological slides in the case of other endocrine tumors and normal tissue.

**DNA Extraction.** Procured cells were resuspended in 30 μl of extraction solution containing 50 mM Tris-HCl, 1 mM EDTA, 0.5% Tween 20, 0.2-0.4 mg/ml proteinase K, pH 8.0, and incubated overnight at 37°C. Following thermal inactivation of proteinase K (95°C for 5 min), 1-1.5 μl of the DNA extract were used for PCR analysis.

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**Table 1** Forty-four lesions from nine FMEN1 patients analyzed for 11q13 LOH

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<tr>
<th>Patient no.</th>
<th>Age and sex</th>
<th>No. of parathyroid glands</th>
<th>No. of regions in parathyroid</th>
<th>No. of ETs duod/pancr/meta (%)</th>
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<td>Total</td>
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<td>55</td>
<td>25</td>
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*ET, endocrine tumor; duod, duodenum; pancr, pancreas; met, metastases.

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**Table 2** LOH on 11q13 in 19 MEN1 parathyroid glands with hyperplasia

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<tr>
<th>Patient no.</th>
<th>PTα gland no./ hyperplastic region no.</th>
<th>D11S956β</th>
<th>D11S480</th>
<th>D11S599</th>
<th>PYGM</th>
<th>D11S4907</th>
<th>D11S4908</th>
<th>PPPICA</th>
<th>INT-2</th>
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α PT-parathyroid.
β Chromosome 11q13 markers are listed in order from centromeric (left) to telomeric (right).
γ —, not informative; •, LOH; ○, retention of heterozygosity; blank - not done or not evaluable.
dNTP (200 pM each), primers (0.1—0.5 @xM each), 0.1 unit Taq DNA polymerase, and standard PCR buffer (Perkin-Elmer Cetus). The reactions were conducted in a total volume of 10 ul that contained 1—1.5 ul of DNA extract, and CA repeat, respectively. Labeling of the PCR product was achieved by GCCTCAAAAACACTCTCCTC-3' and 5'-TCACCCTCTGGT CATCAT

D11S4908, are within 110 kb of each other and D11S460 and are located on gastrin, positive for gastrin by immunostain, clinically gastrinoma; NF, clinically nonfunctional, positive for pancreatic polypeptide by immunostain.

HpaII-digested tumor DNA generated a single fragment (upper or lower band) parathyroid region was considered to be monoclonal if PCR amplification from HpaII (BRL Life Technologies, Gaithersburg, MD) and PCR amplified with primers to HUMARA following previously published conditions (24). The X-chromosome inactivation was obtained from the same microdissection procedure as for LOH. The extracted tumor and normal DNA was digested with X Chromosome Inactivation Analysis. Seven hypercellular parathyroid glands with 16 histologically discrete regions in three female patients were studied for X-chromosome inactivation to assess clonality. The DNA used for the data were reproducible.

Detection of LOH. The eight polymorphic DNA markers used in the study included six published markers, D11S956, D11S480, PYGM (CA)(GA), INT2 (21), PPIACA (22), and D11S599 (23). The two new markers, D11S4907 and D11S4908, are within 110 kb of each other and D11S460 and are located on the same 110-kb PAC clone. The primers for markers D11S956 (5'-ACTGCCTCAAAAACACTCTCCTC-3' and 5'-TCACCCTCTGGT CATCATCCTCCTC-3') and D11S4908 (5'-TGTTGAAATAATGTATGTAGGTTTG-3' and 5'-CTGCGGGCTATTCGTCTTGTC-3') amplify a (CT)(CA) and CA repeat, respectively. Labeling of the PCR product was achieved by either incorporating [α32P]dCTP or using [γ32P] end-labeled primers. PCR was conducted in a total volume of 10 µl that contained 1—1.5 µl of DNA extract, dNTP (200 µM each), primers (0.1—0.5 µM each), 0.1 unit Taq DNA polymerase, and standard PCR buffer (Perkin-Elmer Cetus). The reactions were performed in a Perkin-Elmer Cetus thermal cycler as follows: denaturation at 94°C for 5 min, 35 cycles of annealing for 45 s, extension at 72°C for 1 min, and denaturation at 94°C for 45 s. Annealing temperatures for the sets of primers were 65°C (D11S959), 60°C (D11S4907, D11S4908), 58°C (D11S599, D11S480, PPIACA, INT2), and 56°C (PYGM). Labeled amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide; 20 mM EDTA; 0.05% bromphenol blue, and 0.05% xylene cyanol). The samples were denatured for 5 min at 95°C and resolved on a 6% polyacrylamide gel. Autoradiography was performed with Kodak X-Omat film (Eastman Kodak, Rochester, NY).

The case was considered informative for a polymorphic marker on 11q13 if normal tissue DNA showed two different alleles (heterozygosity). The intensities of the two alleles in the tumor were compared. The complete or near complete (90% decreased intensity) absence of an allele on acrylamide gel was consistent with an inherited, recessive tumor suppressor gene. On the basis of the minimal region of overlapping deletions on 11q13, the MEN1 gene boundaries were placed between markers PYGM (patient 4) and INT2 (patient 8).

The case was considered informative for a polymorphic marker on 11q13 if normal tissue DNA showed two different alleles (heterozygosity). The intensities of the two alleles in the tumor were compared. The complete or near complete (90% decreased intensity) absence of an allele on acrylamide gel was interpreted as LOH (Figs. 1—4). Each of nine patients was informative for at least two markers tested. Each experiment was repeated two or three times, and the data were reproducible.

X Chromosome Inactivation Analysis. Seven hypercellular parathyroid glands with 16 histologically discrete regions in three female patients were studied for X-chromosome inactivation to assess clonality. The DNA used for X-chromosome inactivation was obtained from the same microdissection procedure as for LOH. The extracted tumor and normal DNA was digested with HpaII (BRL Life Technologies, Gaithersburg, MD) and PCR amplified with primers to HUMARA following previously published conditions (24). The parathyroid region was considered to be monoclonal if PCR amplification from HpaII-digested tumor DNA generated a single fragment (upper or lower band) as compared to two fragments of equal intensity generated from HpaII-digested template derived from normal tissue (Fig. 2B; Table 4).

RESULTS

Tissue microdissection yielded reliable DNA procurement from 44 tumors (80 samples) in nine unrelated patients with MEN1. Genomic DNA was extracted from tumor and normal tissue and used for PCR amplification with eight polymorphic DNA markers on 11q13 in the area of the putative MEN1 gene (flanking markers D11S956 and INT2) and one marker on the X chromosome. The LOH result with marker D11S956 in a parathyroid lesion of patient 8 is shown in Fig. 1D. As compared to normal tissue (N), the lower allele is completely lost in region 1 (1.1), whereas in the same region microdissected without removal of the endothelium, the lower allele is still partially present (1.1*). Thus, removal of endothelial contamination yielded more definitive LOH scoring.

Results of the LOH on 11q13 in 19 MEN1 parathyroid glands are summarized in Table 2. For each parathyroid gland studied, at least two polymorphic markers were informative. All parathyroid glands showed LOH on 11q13 (100%), and allelic loss was detected in 50 of 55 precisely microdissected regions with both nodular and diffuse hyperplasia. Three regions in patient 3 (1.2, 4.2, and 4.4) and two regions in patient 5 (2.2 and 2.3) showed retention of heterozygosity at all informative markers tested. All parathyroid regions from each patient showed loss of the same allele (Figs. 2A and 3). LOH data combined with pedigree analysis in patient 4 indicated loss of the wild-type allele derived from the unaffected parent (data not shown), consistent with an inherited, recessive tumor suppressor gene. On the basis of the minimal region of overlapping deletions on 11q13, the MEN1 gene boundaries were placed between markers PYGM and INT2 (patient 9) and INT2 (patient 8).

Results of LOH on 11q13 in 25 MEN1 endocrine tumors of the pancreas, duodenum and their metastases are summarized in Table 3. Sixteen tumors did not show LOH for any of the loci on 11q13 examined. Five of six (83%) pancreatic tumors, including one metastasis, in two patients showed LOH in the area of the putative MEN1 gene. Four of 19 (21%) duodenal gastrinomas and metastases in three

TABLE 3 LOH on 11q13 in 25 MEN1 endocrine tumors of the pancreas and duodenum and their metastases

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Tumor no.</th>
<th>Tumor type</th>
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<th>D11S956</th>
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<th>PYGM</th>
<th>D11S4907</th>
<th>D11S4908</th>
<th>PPIACA</th>
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* Chromosome 11q13 markers are listed in order from centromeric (left) to telomeric (right).

* LOH; O, retention of heterozygosity; —, not informative; blank, not done or not evaluable; LN, lymph node; insulin, positive for insulin by immunostain, clinically insulinoma; gastrin, positive for gastrin by immunostain, clinically gastrinoma; NF, clinically nonfunctional, positive for pancreatic polypeptide by immunostain.

3 P. Manickam et al., manuscript in preparation.
patients demonstrated 11q13 LOH (Fig. 4). All endocrine tumors from each patient with LOH showed loss of the same allele and demonstrated deletions spanning almost the entire MEN1 gene region on chromosome 11q13. In patient 8, in whom all four parathyroid glands demonstrated LOH (Table 2), three gastrinoma metastases showed retention of heterozygosity when tested with the same polymorphic markers (Table 3).

Sixteen regions with 11q13 deletions in seven parathyroid glands in three female MEN1 patients were additionally analyzed for X-chromosome inactivation using PCR amplification for a highly polymorphic region of the HUMARA gene (Ref. 24; Table 4). The X-chromosome inactivation method is based on the fact that in the female either maternally or paternally derived X chromosome is randomly and permanently inactivated. Thus, normal tissue in females is composed of cellular mosaics differing in which of the two X chromosomes has been inactivated. In contrast, in tumors of females, there is a uniformity in the pattern of X-chromosome inactivation that indicates clonality of the cellular composition. Separate hypercellular areas dissected from the same parathyroid gland revealed different clonality patterns (Table 4; Fig. 2B). For example, in patient 2, four nodules from the same gland showed a single clone with the lower allele retained in each region, and one nodule contained two clones. In patient 5, each of the two different regions (3.1 and 3.3) from the same parathyroid gland revealed a single but different clone (Fig. 2B). In patient 8, one region from gland 4 (4.2) contained a single clone, and another region (4.1) showed several clones (Table 4).

**DISCUSSION**

Genetic linkage analysis has located the MEN1 gene between markers D11S480 and D11S460 on chromosome 11q13 (25), and recent tumor allelic deletion mapping data has placed the putative MEN1 gene boundaries between markers PYGM and D11S146 (9, 14). Because MEN1 is rare and the availability of fresh frozen tumors from FMEN 1 patients is limited, we used multiple archival tumors from individual patients for deletion mapping at the MEN1 gene locus. Among the different patients and within the same patient, the LOH rates of parathyroid lesions (100%) and endocrine tumors of the pancreas (83%) were strikingly different from the LOH rate of gastrinomas and metastases (21%). All tumors with LOH showed deletions spanning the entire chromosome 11q13 region. LOH analysis of two tumors in our study placed the proximal MEN1 gene boundary at PYGM and telomeric boundary at INT2 (Table 2). We conclude that multiple archival tumors showing differential LOH patterns in individual MEN1 patients present a valuable resource for tumor suppressor gene mapping.

In this study, a much lower rate of LOH (21%) was detected in gastrinomas of duodenum and metastases than in parathyroid and
pancreatic lesions. Duodenal and pancreatic primary and metastatic tumors with deletions were similar to the tumors with retentions with respect to size and histology (Fig. 4). Only 15 MEN1-associated tumors of the pancreas and duodenum were previously studied for 11q13 LOH (4, 8, 9, 11–15). LOH was detected in 11 pancreatic endocrine tumors (nongastrinomas; Refs. 4, 8, 9, 13–15), and retention of heterozygosity was reported in 3 duodenal gastrinomas (11, 12). Only 4 of 19 gastrinomas in the current study (Table 3) showed LOH with markers tested. In an extended study of MEN1 and sporadic gastrinomas4 we report a slightly higher 11q13 LOH rate for MEN1 gastrinomas (41%; 14 of 34 tumors). Low incidence of LOH on 11q13 in MEN1-associated gastrinomas suggests that these tumors could arise due to inactivation of the wild-type allele via point mutations or small deletions rather than via a loss of large segment of chromosome 11. Identification of the MEN1 gene will allow testing of this possibility. It is important to recognize that gastrinomas are the only common MEN1-associated tumors with a significant malignant potential. Therefore, in addition to initial inactivation of the MEN1 gene, further genetic alterations are probably necessary for development and progression of gastrinomas. Because the clinical incidence of gastrinomas in MEN1 is lower than of parathyroid lesions but the malignant potential is much higher, these tumors offer a unique opportunity to

Fig. 3. LOH result with marker D11S480 in four different nodules from the same parathyroid gland in patient 3. Loss of the upper allele is seen in nodules 4.1 and 4.3, whereas nodules 4.2 and 4.4 show retention of heterozygosity as compared to normal lymph node tissue (N).

Fig. 4. A multiple duodenal gastrinomas in patient 6 (H&E; ×20). Tumor 2 (thin arrow) showed retention of heterozygosity with all markers tested, and tumor 3 (thick arrow) showed a large deletion on 11q13. B, LOH results in 14 gastrinomas in patient 6 with marker D11S480. Compared to normal mucosa (N) the lower allele is lost in tumors 3, 7, 10, and 12 and retained in all other tumors. C, LOH results in the same 14 gastrinomas from patient 6 with marker D11S4907. Compared to normal mucosa (N) the upper allele is lost in tumors 3, 7, 10, and 12 and retained in all other tumors.

study a chain of genetic events necessary for development of malign
nant neoplasm in a setting of familial syndrome.

The pathogenesis of multigland parathyroid disease, currently re
ferred to as parathyroid hyperplasia, is unknown (26). Controversy
exists over whether, in sporadic hyperparathyroidism, such multiglan
dular involvement represents true polyclonal expansion (27, 28) or
whether initially polyclonal hyperplasia evolves into a monoclonal
neoplasm (7, 29, 30). Parathyroid hyperplasia of several parathyroid
glands (90–97%) is the most common finding in MEN1 patients.
Patients who initially present with single-gland disease are rarely
cured by the initial surgery and are likely to develop multigland
disease by age 50 (16), as well as a recurrence in parathyroid aut
ograft. These clinical observations suggest that the pathogenesis of
parathyroid disease in MEN1 involves an abnormality affecting mul
tiple cells in the target tissue.

The molecular investigation of primary parathyroid disease is complex
due to the current definitions of parathyroid “neoplasia” and “hyperpla
sia.” Neoplasm or “parathyroid adenoma” is diagnosed when a single
gland shows enlargement and hypercellularity. The clinicopathological
term of “parathyroid hyperplasia” is applied when two or more glands in
the same patient are abnormal in size and histologically show increased
cellularity. However, the high rate of 11q13 LOH detected in FMEN1
parathyroid lesions suggests that they may be better classified as neo
plastic rather than hyperplastic. We attribute the high LOH rate (100%)
in MEN1 parathyroid glands in our study, as compared to the previously
reported rate of 63% (6–12), to careful histological examination of the
tissue, selection of clearly defined hypercellular areas free of fat and
normal stromal tissue, and immunostain-guided microdissection used to
avoid endothelial contamination.

Our study is the first one to address the origin of MEN1 parathyroid
disease by combining 11q13 LOH and X-chromosome inactivation
data. Because parathyroid glands in MEN1 patients histologically are
composed of nodules or sheets of chief or oxyphil cells (Fig. 1), we
subdivided each parathyroid gland into multiple regions for LOH anal
ysis. Allelic loss on 11q13 was detected in all 19 parathyroid glands in the study. However, five parathyroid glands showed sepa
rate regions within the same gland with different LOH patterns (Table
2). These LOH results suggest that parathyroid lesions are neoplasms
that can consist of more than one tumor clone.

The finding of more than one neoplastic clone within a single
parathyroid gland has important implications for tumor deletion map
ning. Homogenization of whole parathyroid glands admixes all tumor
clones present within the gland. If two of the clones differ with respect
to LOH at a particular marker, the homogenized tissue will result in
equivocal scoring for LOH. For example, in parathyroid gland 4 in
patient 3 (Table 2; Fig. 3), two separate regions show LOH, whereas
two other regions demonstrate retention of heterozygosity. Microdis
section of discrete nodules or regions within the same gland improves
the likelihood of analyzing a single neoplastic clone.

To follow up on the LOH observation, we performed an X-chro
some inactivation analysis (HUMARA; Ref. 24) to assess clonality
of seven glands in three female patients. The DNA used for X-chro
some inactivation and LOH was obtained from the same immu
nostain-guided microdissection procedure, which insured a high per
centage of recovery of tumor cells and minimized normal cell contamina
tion. X-chromosome inactivation study revealed that three of the
seven glands contained more than one clone (Table 4). The fact that
two separate hypercellular regions within parathyroid gland 3 in
patient 5 (Fig. 2B) demonstrated two different tumor clones provides
the first direct genetic evidence that multiple tumors can develop
independently within the same parathyroid gland.

Friedman et al. (7) suggested a “monoclonal” origin for FMEN1
parathyroid tumors based on 11q13 LOH studies. LOH on 11q13 was
seen in the larger FMEN1-associated parathyroid lesions, thus the
authors suggested that “clonal” overgrowth might arise in the context
of generalized hyperplasia in FMEN1 parathyroid tissue. Our present
study shows that in a FMEN1 parathyroid gland, LOH does not
necessarily reflect a monoclonal composition of the tumor. Although
we achieved a 100% LOH rate in MEN1 parathyroid lesions support
ning the neoplastic process, the X-chromosome inactivation data indi
cate that FMEN1 parathyroid neoplasms can be multifocal in origin.

We propose that more neoplastic, “adenomatous” clones develop
in parathyroid tissue, creating a histological appearance of nodules or
sheets, which grow, coalesce, and eventually replace the entire para
thyroid gland (Fig. 1A). In contrast, multiple neoplastic clones in
duodenum (confirmed by X-chromosome inactivation) and pancreas
do not coalesce and histologically present as discrete nodules, “mi
croadenomas,” surrounded by normal tissue (Fig. 4).

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Allelic Deletions on Chromosome 11q13 in Multiple Tumors from Individual MEN1 Patients

Irina A. Lubensky, Larisa V. Debelenko, Zhengping Zhuang, et al.


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