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Research Article

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A Novel Pancreatic Endocrine Tumor Suppressor Gene Locus on Chromosome 3p with Clinical Prognostic Implications

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Abstract

The molecular pathogenesis of pancreatic endocrine tumors is largely unknown. Such tumors are more likely to develop in individuals with the von Hippel-Lindau (VHL) syndrome. We sought to determine whether allelic loss of the recently identified *VHL* tumor suppressor gene on chromosome 3p25-26 occurs in the more common sporadic forms of these tumors. Allelic loss on chromosome 3p was identified in 33% of 43 patients with endocrine tumors of the pancreas. The smallest common region of allelic loss, however, centered not at the *VHL* locus, but rather at 3p25, centromeric to *VHL*. Furthermore, no mutations of the *VHL* gene were identified in these tumors. Loss of alleles on chromosome 3p was associated with clinically malignant disease, whereas tumors with retained 3p alleles were more likely to be benign. Thus, the *VHL* gene does not appear to play a pathogenic role in the development of sporadic pancreatic endocrine tumors. Instead, a locus at chromosome 3p25 may harbor a novel pancreatic endocrine tumor suppressor gene, and allelic loss of this chromosomal region may serve as a molecular marker that helps distinguish benign from clinically malignant disease. (*J. Clin. Invest.* 1997. 100:404–410.) Key words: islet cell tumor • pancreatic endocrine tumor • chromosome 3p25 • VHL gene • tumor suppressor gene

Introduction

Pancreatic endocrine tumors (PETs)[1] represent a distinctive group of malignancies. Frequently associated with unique clinical syndromes that result from an excess of endogenously produced islet hormones such as insulin or glucagon, these tumors are found in as many as 1.5% of patients in autopsy series (1). Though they often behave in a benign manner, they can present with widely metastatic disease. The one feature that separates benign from malignant disease is the absence or presence of distant metastases. Cytologically, benign tumors are indistinguishable from malignant neoplasms. Thus, prognosis cannot reliably be determined when only a primary pancreatic lesion is present.

With certain tumor types, molecular genetic markers have demonstrated a superior ability to determine prognosis when compared with conventional histology. Two key examples include chromosome 18q allelic loss in colorectal cancer, and chromosome 1p loss in neuroblastomas, both of which predict prognosis more accurately than conventional staging techniques (2, 3). Our understanding of the molecular pathogenesis of PETs, however, is quite poor. Only rarely have mutations been identified in either the *ras* oncogene (4–6) or the *p53* tumor suppressor gene (5, 7), the two most commonly mutated oncogenes and tumor suppressor genes in human neoplasia. Inherited familial syndromes can often provide pivotal genetic clues. For example, patients with the multiple endocrine neoplasia type 1 (MEN-1) syndrome frequently develop PETs. The susceptibility gene for the MEN-1 syndrome has been localized to chromosome 11q13, and allelic loss of 11q13 has been identified not only in MEN-1–associated tumors, but also in up to 30% of sporadic PETs (8).

Another familial cancer syndrome that increases the susceptibility to PETs is the von Hippel-Lindau (VHL) syndrome. It is inherited in an autosomal dominant manner, and affected kindreds frequently develop renal cell cancers, pheochromocytomas, retinal angiomas, central nervous system (CNS) hemangioblastomas, and also PETs. The *VHL* gene has been cloned recently, and has been localized to the telomeric end of the short arm of chromosome 3, at 3p25-26 (9). The VHL protein can interact with the elongin family of proteins, and may function as a regulator of transcriptional elongation (10). Renal cell cancer is one of the most common tumors seen in the VHL syndrome, and loss of heterozygosity (LOH) at the *VHL* locus is observed not only in VHL syndrome-associated, but also in sporadic forms of these tumors (11, 12). Consistent with the two-hit biology of tumor suppressor genes, mutations within the three coding exons or exon–intron junctions have been consistently identified in the remaining allele (12–14). Somatic *VHL* gene mutations also have been identified in sporadic CNS hemangioblastomas (15), another tumor type associated with the VHL syndrome, but there has not yet been a systematic examination of the role of the *VHL* gene in PETs.

Methods

Tumor samples and preparation of genomic DNA

48 surgically resected samples from 43 patients with PETs at the Massachusetts General Hospital were collected. No patient had the VHL syndrome. 16 specimens were obtained immediately postoperatively, and were frozen at −80°C. The remaining samples were formalin-fixed, paraffin-embedded archival specimens. Peripheral blood leukocytes or paraffin-embedded nontumor tissue was obtained as a

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1. *Abbreviations used in this paper:* LOH, loss of heterozygosity; MEN-1, multiple endocrine neoplasia type 1; PET, pancreatic endocrine tumor; PTHrP, PTH-related peptide; VHL, von Hippel-Lindau.

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source of control DNA. Genomic DNA from peripheral leukocytes and snap-frozen or paraffin-embedded tissue was isolated as previously described (16, 17). The tumors were classified as functional or nonfunctional on the basis of their clinical presentation and/or serum hormone levels. Histologically, the tumors all displayed the characteristic morphology of neuroendocrine tumors by hematoxylin and eosin staining. In equivocal cases, further confirmation was obtained either with electron microscopy, staining for neuroendocrine markers such as neuron-specific enolase, chromogranin A, synaptophysin, and leu-7, and/or immunohistochemistry for specific islet hormones (including insulin, gastrin, glucagon, somatostatin, vasoactive intestinal polypeptide [VIP], pancreatic polypeptide, and serotonin). Immunohistochemistry for specific hormones is not routinely performed, as staining for multiple hormones is common, and staining patterns do not necessarily reflect the clinical syndrome observed (18). Samples were collected in accordance with regulations of the institutional review board on human studies.

Analyses of allelic loss

Microsatellite markers. Microsatellite markers D3S1317, D3S1038, D3S1110, and D3S1339 (all Research Genetics, Huntsville, AL) on chromosome 3p were PCR-amplified under the following conditions. The D3S1317 and D3S1038 markers were amplified with a 30-s denaturation step at 94°C, a 30-s annealing step at 58°C, and an extension step of 72°C for 45 s with a $MgCl_2$ concentration of 1.5 mM. Markers D3S1339 and D3S1110 were amplified with an annealing temperature of 60°C and a $MgCl_2$ concentration of 2.0 mM. Samples were amplified for 25–30 cycles, followed by a final 5-min elongation at 72°C. Some paraffin-embedded samples required 35 cycles of amplification. 1.25 pmol of the forward primer was ^{32}P-endlabeled, and the reaction was performed in a volume of 20 μl with 5 pmol of the unlabeled reverse primer and 1.0 U of Taq polymerase (Perkin-Elmer Corp., Norwalk, CT) in a buffer containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 200 μM of each deoxynucleotide triphosphate, and 0.01 μg/ml BSA. The samples were then separated on a denaturing 6% polyacrylamide gel with 32% formamide, and autoradiography was performed. Allelic loss was scored as previously described (19).

RFLPs. The RAF-1 RFLP was analyzed by Southern blotting using standard techniques described earlier (20). TaqI or BglI (both Promega Corp., Madison, WI) digests of 10 μg of genomic DNA were probed with a ^{32}P random-prime–labeled (Amersham Corp., Arlington Heights, IL) RAF-1 DNA fragment (American Type Culture Collection, Rockville, MD). Two PCR-based RFLPs were also analyzed (D3F15S2 and VHL nt 19). The D3F15S2 marker (Research Genetics) was amplified as described (21), and was digested with HindIII (Promega). The products were visualized with ethidium bromide after separation in 3% Metaphor agarose gels (FMC Bioproducts, Rockland, ME). The VHL nt 19 RFLP was amplified with primers F1 (forward: 5′ ACAGTAACGAGTTGGCCTAG) and R2 (reverse: 5′ CTGCGTGCGCGCTCCCGAGT) as previously described (22). [α^{32}P]dATP (NEN Research Products, Boston, MA) was incorporated into the PCR products, which were then digested with HaeIII (Promega), separated on a 10% nondenaturing polyacrylamide gel, and visualized with autoradiography.

VHL gene sequencing

The coding exons and the intron–exon junctions of the *VHL* gene were sequenced by a combination of two methods. Exons 2 and 3 were PCR-amplified with the respective primer pairs (12): No. 102 (forward: 5′ CTTTAACAACCTTTGCTTGTCCCGATA)/No. 103 (reverse: 5′ GTCTATCCTGTACTTACCACAACACCT) and No. 107 (forward: 5′ CTGAGACCCTAGTCTGCCACTGAGGAT)/ No. 6 (reverse: 5′ CAAAAGCTGAGATGAAACAGTGTAAGT). PCR cycle sequencing (Stratagene Inc., La Jolla, CA) was then performed in both directions with primer Nos. 102, 107, and 6. An internal primer No. 103-1 (5′ TGGCAAAAATAGGCTGTC) was used in place of primer No. 103. The coding sequences of exon 1 were PCR-amplified with primer Nos. 3 (forward: 5′ GCGGCGTCCGGCCCGGGTGGTCTGGAT) and 101 (reverse: 5′ TGGGTCGGGCCTAAGCGCCGGGCCCGT), or in three overlapping segments with primers Nos. 3 and 8 (reverse: 5′ CCTCGGCGCCCGACTCCTCCCCGCCGT), No. 1 (forward: 5′ GAGGCAGGCGTCGAAGAGTACGGCCCT), and No. 10 (reverse: 5′ GACTGCGATTGCAGAAGATGACCTGGG), and No. 9 (forward: 5′ CATCTTCTGCAATCGCAGTCCGCGCGT), and No. 101, all with the addition of DMSO. Exon 1 products were subcloned into the pCRII vector (Stratagene), and at least five independent clones were sequenced using the dideoxynucleotide chain termination method with Sequenase™ (United States Biochemical Corp., Cleveland, OH). Primers were all synthesized on a DNA synthesizer (Applied Biosystems, Inc., Foster City, CA).

Statistical analysis

Correlations between LOH on chromosome 3p and clinical indices were analyzed using Fisher's exact test. For variables that were significant in univariate analysis, multiple logistic regression analysis was performed. $P < 0.05$ was considered statistically significant.

Results

Patients and tumor samples. 48 tumor samples were collected from a total of 43 patients. Multiple samples were obtained from four patients: one patient with the MEN-1 syndrome had three pancreatic tumors excised simultaneously, one patient had a primary tumor, and 8 yr later had a retroperitoneal recurrence resected, one patient with disseminated disease had the primary lesion resected in addition to hepatic metastases 9 yr later, and one patient had a primary tumor removed in addition to a hepatic recurrence 18 mo later.

Among the 43 patients, 20 were male and 23 were female. The mean age at the time of surgical resection among the men was 52.5 yr (±17.0) with a range of 17–75 yr. For the women, the age range was similar (25–80 yr) with a mean of 54.1 yr (±16.0).

22 patients had disease that was localized to the pancreas, and 21 had more advanced disease, defined by extrapancreatic spread to surrounding soft tissue, lymph nodes, and/or the liver. Distant hepatic metastases were present in 15 of the 21 advanced cases. One primary tumor (gastrinoma) from a patient with advanced disease was located in the periampullary region (case no. 38). Four patients had PETs in association with the MEN-1 syndrome.

Among the total 48 tumor samples, insulinomas were the most common type, comprising 17 of the cases. There were nine gastrinomas, two glucagonomas, two ACTH-producing tumors, one serotonin-producing carcinoid, and three tumors associated with hypercalcemia and low PTH levels, presumably secreting PTH-related peptide (PTHrP). No VIPomas or somatostatinomas were identified. 14 cases were classified as nonfunctional by clinical criteria. Immunohistochemical studies for specific islet hormones were performed in the 12 nonfunctional cases for which adequate tissue was available. Staining patterns were positive in only two cases, both for pancreatic polypeptide (PP). Finally, in two cases, the hormonal function of the tumor was observed to change over time; nonfunctional hepatic metastases from a primary insulinoma were resected 9 yr after the initial diagnosis (case No. 2), and one nonfunctional tumor recurred locally 8 yr later as a PTHrP-producing tumor (case No. 32).

Allelic LOH on chromosome 3p. Seven polymorphic markers within and flanking the *VHL* gene on chromosome 3p25-26

Table I. Polymorphic Markers Used for Analysis of Allelic Loss on Chromosome 3p

Chromosomal location	Marker name	Marker type
3p25-26	VHL Nt 19	PCR-RFLP
3p25	D3S1317	Microsatellite
	D3S1038	Microsatellite
	RAF-1	RFLP
	D3S1110	Microsatellite
3p21	D3F15S2	PCR-RFLP
	D3S1339	Microsatellite

The seven polymorphic markers are listed in order from telomeric to centromeric, and the type of polymorphism is indicated at the right. The order of the markers is determined from a combination of genetic and physical maps of chromosome 3p (11, 48–50).

were selected. The markers represent a combination of microsatellite and RFLP markers, and are listed in Table I. The intragenic basepair polymorphism at nucleotide 19 (nt 19) of the *VHL* gene was first analyzed. Only 15 of the 43 patients were heterozygous (informative) at this locus, and tumors from 3 of these 15 patients (20%) demonstrated allelic LOH. Markers at 3p25 flanking the *VHL* gene (D3S1317, D3S1038, RAF-1, and D3S1110) were then examined. 42 of the 43 patients were heterozygous (informative) at one or more of these markers. Tumor samples from 13 patients (31%) demonstrated LOH at one or more of these 3p25 loci. Representative examples of tumors with LOH are shown in Fig. 1.

Because chromosome 3p21 has been identified as a region that may harbor a tumor suppressor gene for many types of cancer including lung and renal cancer (23–26), we extended our analysis of tumor samples to include 3p21. 34 of the 43 patients were informative. 9 of these 34 patients (26%) had tumors with allelic loss at 3p21. In eight of these nine tumors, this allelic loss at 3p21 appeared to be part of a larger chromosomal deletion that also encompassed alleles at 3p25. Not all tumors with 3p25 LOH, however, had allelic loss that extended to 3p21. Tumors from three patients had allelic loss that was confined to 3p25. One gastrinoma (case No. 40) was identified with allelic loss at 3p21 without apparent loss at the markers previously tested at 3p25. That patient, however, was informative only at the most telomeric 3p25 markers.

Overall, all 43 patients were informative at one or more of these distal chromosome 3p markers. Tumors from 14 of the 43 patients (33%) demonstrated LOH at one or more of these seven markers on chromosome 3p. Fig. 2 summarizes the allelotyping data for these 14 cases. In some tumors from patients such as No. 2 or No. 32, there was a large region of deletion with allelic loss at all informative 3p25 markers. In tumors from other patients such as No. 17 or No. 34, however, allelic loss was confined to a single marker. When all these cases are analyzed collectively, the smallest common region of overlapping deletion centers not at the *VHL* gene on 3p25-26, but rather more centromeric at 3p25, between the markers RAF-1 and D3S1110.

In patients with more than one tumor sample available, the genetic profiles were identical at the 3p loci tested. For example, three samples were obtained simultaneously from a patient with the MEN-1 syndrome (case No. 37). The patient was informative at only one marker (D3S1317), and all three samples retained heterozygosity. Two tumor samples were obtained from patient No. 32: a primary tumor and a retroperitoneal recurrence 8 yr later. LOH was detected at all 3p markers that were analyzed in both samples. Similarly, tumor samples from primary resections and reexplorations for recurrent disease 9 and 1.5 yr later were obtained from patients No. 2 and No. 4, respectively, and the profile of LOH on 3p was identical.

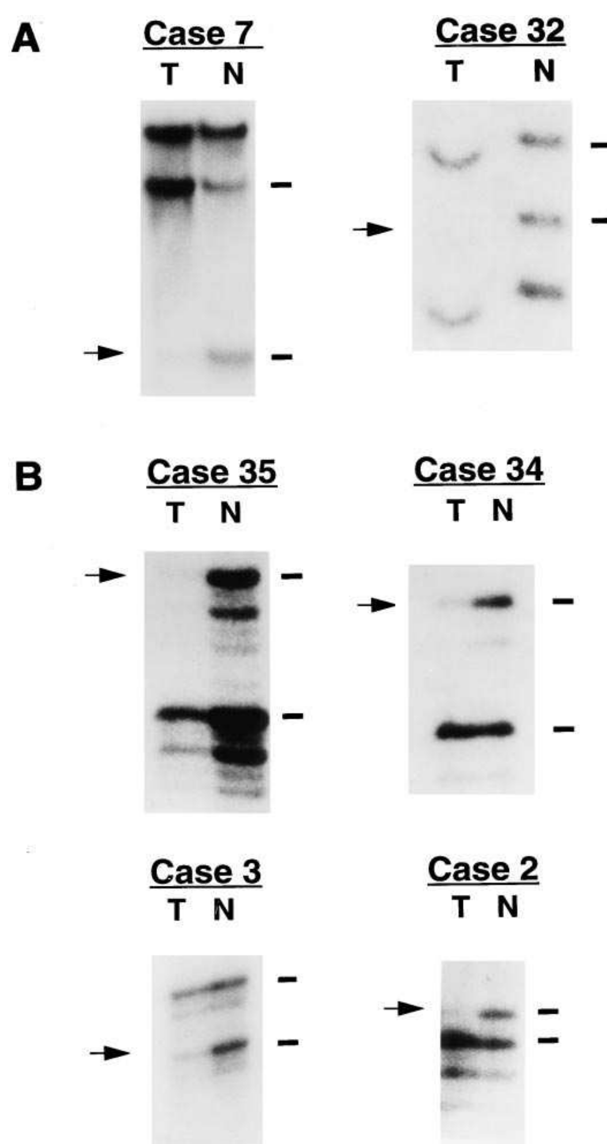

Figure 1. Representative examples of tumor-specific allelic loss on chromosome 3p. All cases illustrated are informative. (*A*) RFLP markers VHL nt 19 (case 7) and RAF-1 (case 32). (*B*) Microsatellite markers D3S1038 (case 35), D3S1110 (case 34), D3S1317 (case 3), and D3S1339 (case 2). *T*, tumor sample; *N*, paired normal control. The positions of the two wild-type alleles are indicated by the dashes at the right, and the arrow indicates the lost allele in the tumor sample. In *A*, a third invariant band is also seen in the tumor sample and the normal control of both cases.

Sequencing of the VHL gene. Although the smallest common region of overlapping deletion in these PETs was located centromeric to the *VHL* gene, many samples demonstrated allelic loss that did extend into the *VHL* gene. For this reason, the three coding exons of the *VHL* gene were sequenced in all

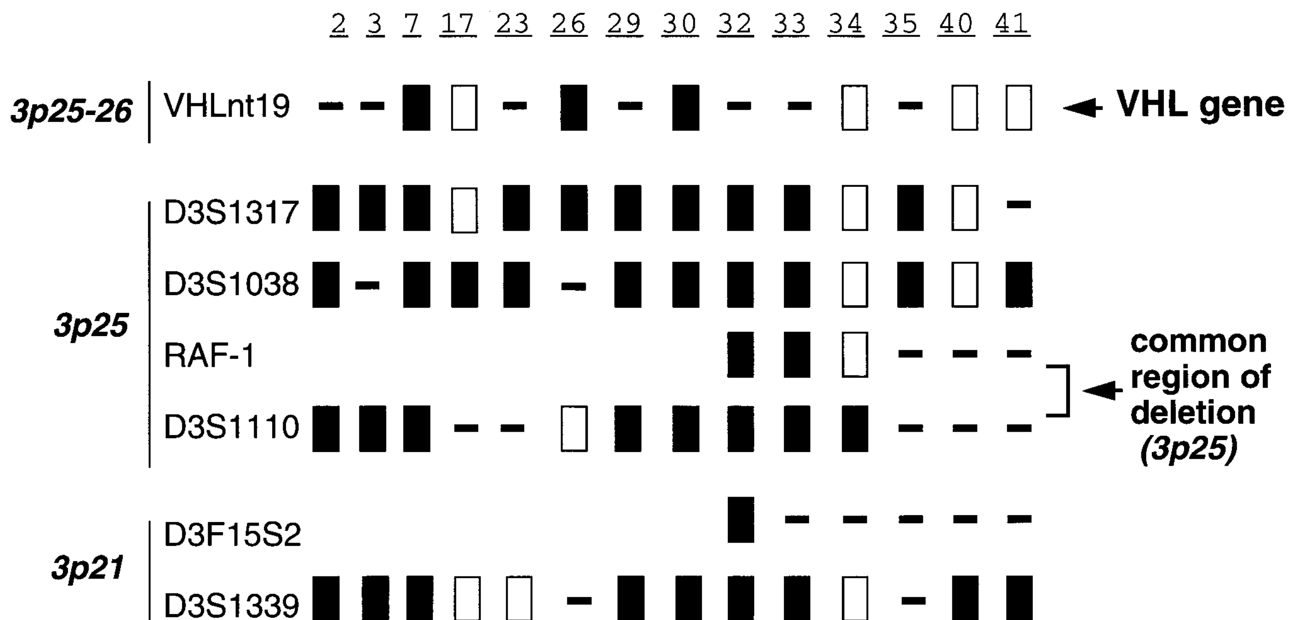

Figure 2. Summary of allelotyping data for chromosome 3p markers. The chromosome 3p markers are listed on the left from telomeric (3p25-26) to centromeric (3p21). The 14 cases are listed from left to right. ■, informative with allelic loss; □, informative with retained alleles; -, uninformative. The VHL nt 19 marker lies within the *VHL* gene. The smallest common region of overlapping deletion at chromosome 3p25 is demarcated at the right by the bracket. In some cases, multiple tumor samples were available from a single patient (cases 2, 4, 32, and 37). In all these cases, the allelotype was identical at the 3p markers tested.

cases with chromosome 3p allelic loss. The primer pairs used for this analysis are schematically illustrated in Fig. 3. All three coding exons and their splice sites were sequenced through a combination of cycle-sequencing and subcloning techniques. In none of the 14 cases with 3p allelic loss was a mutation in the *VHL* gene identified.

Clinical correlates of chromosome 3p allelic loss. We sought to determine if chromosome 3p allelic loss correlated with any clinical feature of these tumors. No association was identified between 3p allelic loss and patient age or sex. When chromosome 3p allelic loss was compared with disease stage, however, a strong correlation was observed ($P = 0.001$), and this correlation remained strong after controlling for age, sex, and tumor type ($P = 0.004$). Details of the 43 cases are summarized in Table II. Among the 21 patients with advanced disease (defined by extrapancreatic spread of tumor), 12 (57.1%) had 3p LOH. Only 9.1% (2/22) of patients with localized disease, however, had such allelic loss. Cases with the poorest prognosis are those with distant hepatic metastases, and among such patients with informative markers at the smallest common region of deletion on 3p25, 75% (9/12) had 3p LOH. In some of these patients, advanced disease was apparent at the time of clinical presentation. Two patients (Nos. 23 and 30), however, appeared to have disease localized to the pancreas at the time of primary resection, but distant hepatic metastases subsequently developed 1 and 4 yr later. Also, two patients (Nos. 7 and 40) had disease judged to be confined to regional lymph nodes, but hepatic metastases were detected 6 mo and 1 yr later. In all four of these cases, 3p allelic loss was present in the original tumor sample. Overall, the positive predictive value of 3p allelic loss for predicting advanced malignant disease was 85.7% (12/14). This value compares favorably to those obtained using hormonal subtype as a predictor of disease stage. Specifically, the positive predictive values of the nonfunctional and gastrinoma subtypes for predicting advanced disease were only 66.7% (8/12 and 6/9, respectively). The positive predictive value of the insulinoma subtype for predicting benign disease was 76.5% (13/17).

No statistical correlation was observed between 3p loss and the insulinoma, gastrinoma, or nonfunctional subtype. Too few glucagonomas, carcinoid tumors, PPomas, and ACTH-producing tumors were studied to make any definitive conclusions. All three PTHrP-producing tumors, however, had loss of chromosome 3p alleles. An association was suggested by univariate

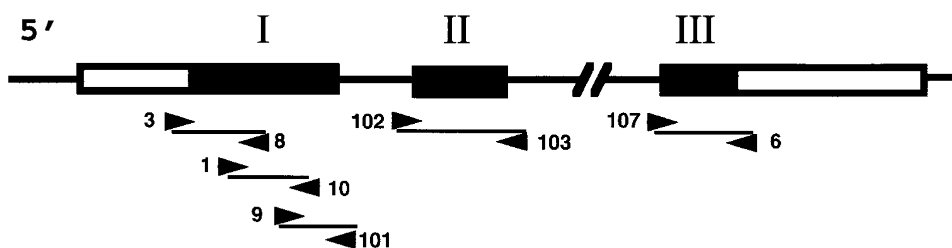

Figure 3. Schematic diagram of primers used for sequencing of the *VHL* gene. The three *VHL* gene exons are illustrated with open rectangles. Primer pairs 3/101, 3/8, 1/10, or 9/101 were used to amplify exon 1. Primer pair 102/103 was used for exon 2, and primer pair 107/6 was used for exon 3. Sequences are described in Methods.

Table II. Disease Stage, Hormonal Function, and 3p LOH in PETs

Patient No.	Disease stage	Tumor samples	Hormonal type	3p LOH
1	Benign	Primary pancreatic	Nonfunctional	−
2	Advanced (H)	Primary pancreatic	Insulin	+
		hepatic recurrence	Nonfunctional	+
3	Advanced (LN, H)	Primary pancreatic	Insulin	+
4	Advanced (LN, H)	Primary pancreatic	ACTH	−
		lymph node recurrence	ACTH	−
5	Benign	Primary pancreatic	Insulin	−
6	Benign	Primary pancreatic	Gastrin	−
7	Advanced (LN, H)	Primary pancreatic	Insulin	+
8	Advanced (H)	Hepatic metastasis	Gastrin	−
9	Advanced (LN, duodenal invasion)	Primary pancreatic	Nonfunctional	−
10	Benign	Primary pancreatic	Insulin	−
11	Benign	Primary pancreatic	Insulin	−
12	Benign	Primary pancreatic	Insulin	−
13	Advanced (LN, H)	Primary pancreatic	Nonfunctional	−
14	Benign	Primary pancreatic	Gastrin	−
15	Benign	Primary pancreatic	Insulin	−
16	Benign	Primary pancreatic	Glucagon	−
17	Advanced (H)	Primary pancreatic	PTHrP	+
18	Benign	Primary pancreatic	Nonfunctional	−
19	Benign	Primary pancreatic	Gastrin	−
20	Benign	Primary pancreatic	Insulin	−
21	Benign	Primary pancreatic	Insulin	−
22	Benign	Primary pancreatic	Insulin	−
23	Advanced (H)	Primary pancreatic	PTHrP	+
24	Benign	Primary pancreatic	Insulin	−
25	Benign	Primary pancreatic	Insulin	−
26	Benign	Primary pancreatic	Insulin	+
27	Benign	Primary pancreatic	Nonfunctional	−
28	Advanced (LN, soft tissue invasion)	Primary pancreatic	Gastrin	−
29	Advanced (LN, H)	Primary pancreatic	Gastrin	+
30	Advanced (H)	Primary pancreatic	Glucagon	+
31	Benign	Primary pancreatic	Insulin	−
32	Advanced (retroperitoneal)	Primary pancreatic	Nonfunctional	+
		retroperitoneal recurrence	PTHrP	+
33	Benign	Primary pancreatic	Insulin	+
34	Advanced (LN, duodenal invasion)	Primary pancreatic	Nonfunctional	+
35	Advanced (H)	Primary pancreatic	Nonfunctional (pancreatic polypeptide)	+
36	Benign	Primary pancreatic	Serotonin	−
37	Advanced (LN)	Primary pancreatic	Gastrin	−
		Primary pancreatic	Insulin	−
		Primary pancreatic	Nonfunctional	−
38	Advanced (LN, retroperitoneal recurrence)	Periampullary	Gastrin	−
39	Advanced (H)	Primary pancreatic	Nonfunctional (pancreatic polypeptide)	+
40	Advanced (LN, H)	Lymph node	Gastrin	+
41	Advanced (LN, H)	Lymph node	Nonfunctional	+
42	Benign	Primary pancreatic	Nonfunctional	−
43	Advanced (H)	Primary pancreatic	Nonfunctional	−

Benign cases are those with disease localized to the pancreas, and advanced cases are defined by extrapancreatic spread of tumor (*LN*, lymph node; *H*, hepatic metastases). Four cases were associated with the MEN-1 syndrome (nos. 24, 25, 26, and 37).

analysis (P = .032), but the number of PTHrP-producing tumors was too small to confirm an association with logistic regression analysis. All three PTHrP-producing tumors were advanced tumors, and their association with 3p LOH could be accounted for by their advanced stage.

Discussion

Little is understood regarding the molecular pathogenesis of endocrine tumors of the pancreas. This report is the first to identify a region, other than the MEN-1 locus on chromosome

11q13, as containing a putative tumor suppressor gene that commonly participates in the molecular pathogenesis of this unique group of pancreatic tumors. Because PETs are part of the spectrum of the inherited VHL syndrome, and because molecular correlations do exist between inherited and sporadic forms of tumors (12, 27), we analyzed the *VHL* tumor suppressor gene in a large number of these tumors. In renal cancer, the most common malignancy in the VHL syndrome, the *VHL* gene is typically inactivated by a point mutation in one allele, and by a large chromosomal deletion in the other (12, 28). Such chromosomal deletions are detectable as allelic loss at polymorphic markers in the region. Thus, PETs from 43 patients were first analyzed for allelic loss at the *VHL* locus. Only 20% of informative cases had such loss, but 33% of cases had loss of chromosome 3p markers that flanked the *VHL* gene. This figure may be an underestimate, as some tumors may harbor microdeletions on 3p that are nestled between the markers tested. In addition, amplification during PCR of genomic DNA from the small population of normal cells present with the tumor specimen may obscure true allelic loss.

Among the distinct tumors, the smallest overlapping region of deletion localized to chromosome 3p25, centromeric to the *VHL* gene. Thus, the true putative tumor suppressor gene target of these deletions appeared to be different from *VHL*. Nonetheless, because the deleted region in some cases did include *VHL*, and because *VHL* was a compelling candidate tumor suppressor gene for PETs, we sequenced the *VHL* gene in all 14 tumors with any 3p allelic loss. No mutations were detected in any of the three exons, or their exon–intron junctions. These results do not exclude the possibility that in some tumors, inactivating mutations or epigenetic changes are present in the noncoding regions of the gene. For example, methylation of the *VHL* promoter has been reported to silence expression of the *VHL* gene (29). For tumor types in which *VHL* is an established tumor suppressor gene, however, and for that matter, for all known tumor suppressor genes, deletion and coding/splice site mutations are the most prevalent mechanisms of gene inactivation (12, 14, 30–34). Thus, our results indicate that clonal inactivation of the *VHL* tumor suppressor gene does not play an important role in the pathogenesis of sporadic PETs. Interestingly, for pheochromocytomas, which can be part of the inherited VHL syndrome, sporadic cases are also characterized by chromosome 3p allelic loss without *VHL* gene mutations (12, 35). Unidentified mutations in *VHL* gene noncoding regions may explain this observation, but more plausible is the involvement of another tumor suppressor gene on 3p.

Many potential tumor suppressor gene loci have been identified on chromosome 3p. In addition to the *VHL* gene at 3p25-26, high frequency allelic loss at chromosome 3p21 in lung cancer suggests the presence of another tumor suppressor gene (24, 26, 36). Several studies of head and neck tumors as well as esophageal squamous cell cancer have also identified 3p25 as a region of frequent allelic loss (37–39). No potential tumor suppressor genes, however, have yet been identified at chromosome 3p25. Genes that have been localized to 3p25 include the histamine H1 receptor (40), oxytocin receptor (41), xeroderma pigmentosum-C (42), and the IL-5 receptor alpha (43), none of which appears to be an attractive candidate as a pancreatic tumor suppressor gene. The putative novel tumor suppressor gene on 3p25 involved in pancreatic endocrine cell tumorigenesis may or may not be the same one implicated in other tumors such as head and neck or esophageal cancer. Certainly, our findings constitute an important first step toward the positional or positional candidate cloning (44) of the key PET tumor suppressor gene; such a gene should be an excellent target for novel therapeutic approaches in the future.

Allelic loss at chromosome 3p25 is correlated with more clinically advanced disease. Such loss may thus serve as a molecular marker that helps to distinguish benign from potentially malignant disease. Currently, there is no histopathologic feature other than the presence of gross metastases that reliably can distinguish benign from malignant disease, and thus, there are no markers that can help predict malignancy when a primary lesion is identified. The alpha subunit of human chorionic gonadotropin (hCG) has been proposed as a potential marker of malignancy (45), but this association has not been confirmed (46, 47). The ability to identify such potentially malignant lesions early may ultimately alter management, as patients with such tumors may be candidates for early adjuvant chemotherapy. Only carefully designed controlled trials can confirm if such an approach will ultimately yield clinical benefit. The finding that advanced lesions are more likely to have allelic loss on chromosome 3p also suggests that the loss of this putative pancreatic endocrine tumor suppressor gene may be associated with the metastatic process. Benign and malignant tumors share similar well-differentiated cytologic features, and are distinguished only by the absence or presence of distant metastases, respectively. Thus, this novel tumor suppressor gene at 3p25 may play a role in suppressing the process of metastasis rather than regulating other aspects of cellular growth or differentiation.

In summary, the *VHL* gene does not appear to play an important role in the molecular pathogenesis of sporadic PETs. Instead, chromosome 3p25 may harbor a novel pancreatic endocrine tumor suppressor gene. Loss of alleles in this region may potentially serve as a molecular marker, helping to distinguish clinically benign from malignant PETs, thereby providing meaningful prognostic information.

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