Mutation of the MENIN Gene in Sporadic Pancreatic Endocrine Tumors

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ABSTRACT

Pancreatic endocrine tumors occur both sporadically and as part of the multiple endocrine neoplasia type 1 (MEN1) syndrome. MEN1 is an autosomal dominant disease characterized by parathyroid hyperplasia, pancreatic endocrine tumors, and pituitary adenomas. The MEN1 gene called MENIN maps to chromosome 11q13 and is thought to function as a tumor suppressor gene. We previously demonstrated loss of heterozygosity (LOH) at 11q13 in ~40% of sporadic pancreatic endocrine tumors and hypothesize that MENIN is involved in the development of these tumors. Thirty-one sporadic pancreatic endocrine tumors were analyzed for mutation of MENIN by nonradioactive single-stranded conformation polymorphism. Twelve mutations were detected in 31 sporadic pancreatic endocrine tumors (34%). Twelve of these 31 tumors previously demonstrated loss of heterozygosity at 11q13. Of the tumors with LOH, seven contained mutations of the MENIN gene (58%). The majority of the MENIN mutations occurred within exon 2. Two independent mutations in MENIN were detected in a gastrinoma that also revealed LOH, leading to the possibility of another tumor suppressor gene locus at 11q13. Mutations were present in both benign and malignant pancreatic endocrine tumors, suggesting that a MENIN gene mutation is a frequent and early event in the tumorigenesis. The high incidence of truncating mutations in tumors with LOH at 11q13 support the hypothesis that MENIN is a tumor suppressor gene.

INTRODUCTION

Pancreatic endocrine tumors are characterized by their histological appearance and the hormones they produce. They are usually sporadic, but they may also arise as part of the MEN13 syndrome (1). The two most common pancreatic endocrine tumors, gastrinomas and insulinomas, produce characteristic syndromes as a result of their hormonal secretions, gastrin and insulin, respectively. Besides these two major tumor phenotypes, other pancreatic endocrine tumors secrete glucagon, somatostatin, or pancreatic polypeptide. Among these various pancreatic endocrine tumors, their malignant potential varies widely. Insulinomas are usually benign and curable, whereas the other tumors have a much higher malignant potential (1-4). Little is known about the molecular genetics of these tumors. The most common abnormality identified to date is LOH at 11q13 (5-11). Both sporadic and familial pancreatic endocrine tumors have LOH overlapping this region. The gene responsible for MEN1, called MENIN, maps to this area and is implicated in the development of these tumors.

MEN1 is an autosomal dominant disease characterized by parathyroid hyperplasia, pancreatic endocrine tumors, and pituitary adenomas (12). Other tumors, such as adrenal adenomas, carcinoids, and lipomas, also arise in certain MEN1 families (13-15). The predisposing gene, MENIN, encodes a 2.7-kb messenger with a predicted protein of 610 amino acids (16-18). Although its precise biochemical function is unknown, it appears to behave as a tumor suppressor gene.

The biological behavior of these tumors varies, however; both benign and malignant tumors demonstrate LOH at 11q13, implicating this chromosome region early in pancreatic endocrine tumor development. It is important to determine the frequency of mutation in these sporadic tumors to clearly define the role of MENIN in sporadic pancreatic endocrine tumors.

We studied sporadic pancreatic endocrine tumors for mutations in the MENIN gene to determine its role in the pathogenesis of these tumors. Our previous studies established a high frequency of LOH in these tumors (5, 8, 19, 20). This study identifies a high frequency of MENIN mutations in these tumors, which supports our hypothesis that the MENIN gene plays a key role in the development of sporadic pancreatic endocrine tumors. Some pancreatic endocrine tumors with LOH at 11q13 did not have mutation of MENIN. In addition, a pancreatic endocrine tumor with LOH at 11q13 also revealed two independent mutations of MENIN, suggesting the possibility of another tumor suppressor gene locus at 11q13 involved with the pathogenesis of these tumors.

MATERIALS AND METHODS

Tumor Samples. Thirty-one sporadic pancreatic endocrine tumors were obtained after operation and immediately snap frozen in liquid nitrogen. The tumors included 21 gastrinomas, 6 nonfunctional tumors, 2 insulinomas, and 2 vasoactive peptideomas. Frozen section analysis confirmed that the tumors were essentially free of adjacent normal tissue. Constitutional DNAs were obtained from peripheral blood leukocytes of each respective patient. DNAs from tumor and normal tissues were isolated by phenol-chloroform extraction (8).

PCR. Primers for SSCP (Table 1) were designed based on the published MENIN sequence (accession U93237; Ref. 16). The 5' and 3' untranslated regions were excluded from analysis. Tumor and corresponding constitutional DNAs were amplified in a final volume of 20 μl consisting of 20 ng of DNA, 1.5 mM MgCl2, 1X PCR buffer, 1 mM deoxynucleotide triphosphate, 1.25 units of Taq polymerase, and TaqStart Antibody (Clontech, Palo Alto, CA) in 96-well format (Perkin-Elmer 9600 thermocycler). Exons 2.1 and 10.1 required optimization with the Advantage GC Genomic PCR kit to obtain a single PCR fragment (Clontech). The DNA was amplified with 40 cycles: initial denaturation at 94°C for 5 min followed by repeated cycles of 94°C for 30 s, Ta for 30 s, and 72°C for 30 s. Products were then diluted 1:1 with a loading dye (90% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, and 20 mM EDTA) and stored at −20°C until used.

Nonisotopic SSCP. Cold SSCP was performed as described previously with minor modifications described below (21). The dilute PCR product was denatured at 85°C for 3 min and then quenched on a dry ice/isopropanol bath. Ten μl of the diluted PCR products were loaded into precast 12% Tris-glycine polyacrylamide gels (Novex, San Diego, CA). The gels were electrophoresed at 200 V in the ThermoFlow Mini-Cell (Novex) kept at constant temperature using a temperature-controlled circulating buffer (Bio-Rad, San Diego, CA). Gels were visualized with SYBR Green I and SYBR Green II DNA stain (Molecular Probes, Eugene, OR). Undenatured normal human DNA and a 100-bp ladder size marker were run on each gel. Aberrantly migrating bands were removed from the gel using a pipette tip. The DNA was allowed to elute into 10 μl of sterile, double-distilled water overnight.

Subcloning and Sequencing of Mutant Bands. The mutant DNA samples were reamplified and directly cloned into pCR 2.1 vector using the TA Cloning kit (Invitrogen, San Diego, CA). Several independent colonies were screened by SSCP analysis and compared with the original mutant DNA. The colonies that reproduced the aberrantly migrating bands were further characterized by sequencing. Plasmid DNA was isolated by anion exchange resin column
Table 1. PCR primer sequences for SSCP analysis

<table>
<thead>
<tr>
<th>MENIN primers</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
<th>TA (°C)</th>
</tr>
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<tbody>
<tr>
<td>Exon 2.1 U</td>
<td>AAC-CTT-AGG-GCA-CCC-TGG</td>
<td>246</td>
<td>65°</td>
</tr>
<tr>
<td>Exon 2.1 D</td>
<td>ACA-GGC-AGG-AAG-TGC-TCC-AC</td>
<td>225</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 2.2 UN</td>
<td>TTT-CCT-TGC-TGC-TGG-GCT-TC</td>
<td>225</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 2.2 D</td>
<td>ACC-CGC-CTT-TGC-AGG-GTA-GA</td>
<td>184</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 2.3 UN</td>
<td>AGA-TCC-GAG-GCG-TCG-AC</td>
<td>270</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 2.3 D</td>
<td>AGA-TCG-CCA-CTC-ATG-GGG-CT</td>
<td>187</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 3 U</td>
<td>GCT-ACT-AGC-ACT-CCT-CTT-CC</td>
<td>293</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 3 D</td>
<td>TTG-GCC-CTC-CTG-AGA-CAT-AA</td>
<td>223</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 4 U</td>
<td>GTG-GGT-GAG-GGG-CTC-CCA</td>
<td>219</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 4 D</td>
<td>GGT-GGG-GCC-TGC-AGA-CAG</td>
<td>271</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 5 U</td>
<td>TCT-GCA-CCC-TCC-TTG-GAT-GC</td>
<td>224</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 5 D</td>
<td>GGA-AGA-AAG-GAC-AGG-CTG-C</td>
<td>226</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 6 U</td>
<td>GAT-GGT-GAG-GGCC-TTC-TCA</td>
<td>233</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 6 D</td>
<td>GGT-GAG-GCC-TGC-AGA-CAG</td>
<td>271</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 7 U</td>
<td>CCC-TTG-CAT-GTC-GGT-GCC</td>
<td>224</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 7 D</td>
<td>GCC-AGA-AGA-AAG-GCT-CTG-T</td>
<td>226</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 8 U</td>
<td>AAA-CGC-GAG-GAG-GCA-AGA</td>
<td>291</td>
<td>60°</td>
</tr>
<tr>
<td>Exon 8 D</td>
<td>GGG-AAC-CTT-AGC-GGA-CCC-TGG</td>
<td>246</td>
<td>65°</td>
</tr>
</tbody>
</table>

RESULTS

Thirty-one sporadic pancreatic endocrine tumors were examined for mutation of the MENIN gene. Previous studies had characterized these tumors for LOH at 11q13 (5, 8, 19, 20). Tumor and corresponding peripheral blood leukocyte genomic DNAs from each patient were amplified and compared by SSCP to identify MENIN mutations (Fig. 1). Twelve of the 31 tumors demonstrated aberrant migrating bands by SSCP (Table 2). The SSCP results were confirmed by reamplifying a second aliquot of DNA. Most of the mutations were identified within gastrinomas. Of the 21 gastrinomas analyzed, 10 (47%) revealed mutation of the MENIN gene. Only 2 of 10 (20%) nongastrinomas revealed mutation. One gastrinoma (8T) had mutations of two alleles, whereas the remaining tumors had mutation of a single allele.

The mutations consisted of eight deletions and four missense mutations. The deletions ranged in size from 1 to 61 nucleotides in length. Two of the deletions (tumors 9T and 66T) are in-frame, resulting in loss of one and three amino acids, within exons 2 and 4, respectively. The remaining deletions cause a frameshift, resulting in truncation of the final protein product. Although the functional significance of missense mutations is dependent on the analysis of the MENIN protein, all of the amino acid substitutions change the characteristic of the R group of the amino acid, uncharged polar to positive charged (Q141R, S145R) and positive charged to nonpolar (R330P). Two tumors, 94T and 152T, contained the same missense mutation of exon 7. Otherwise, all of the mutations were unique.

Mutations were found in exons 2, 3, 4, 7, and 8. Although mutations occurred throughout the open reading frame, exon 2 exhibited a high propensity for mutations, harboring 7 of the 12 mutations. Three of the exon 2 mutations resulted in deletion of an amino acid or a nonconservative amino acid substitution, whereas the other four resulted in protein truncation. Of the 31 sporadic tumors studied, 12 had LOH at 11q13, and mutations were detected in 7 of these tumors. Five tumors have not demonstrated LOH but revealed mutation of a single MENIN allele. Two independent mutations in exon 2 were found in one tumor, 8T, which also demonstrated LOH.

DISCUSSION

The MENIN gene is responsible for the MEN1 syndrome (16–18). Approximately 80% of patients with MEN1 have clinically important pancreatic endocrine tumors, with gastrinomas being the most common. There does not appear to be a correlation between the tumor phenotype and the patient’s particular germ-line mutation. There are, however, mutation hotspots in the MENIN gene in codons 83/84, 210/211, and 516 (18). Of the 31 sporadic pancreatic endocrine tumors analyzed in this study, 12 (39%) revealed mutation of the MENIN gene. It is hypothesized that this gene functions as a tumor suppressor because there is a high frequency of LOH overlapping this locus in both familial and sporadic tumors, and the majority of germ line and tumor mutations result in protein truncation. From our results, pancreatic endocrine tumors with LOH have a higher incidence of mutation of the MENIN gene compared with tumors without LOH.

Five tumors with LOH at 11q13 did not demonstrate mutation of the MENIN gene. These results confirm the findings of a recent study wherein there was a relatively high frequency of pancreatic endocrine...
expected that the remaining tumors, mostly nonfunctional, would have a higher frequency of mutation. It may be that these tumors do not have involvement of the MENIN gene in their development. This is somewhat surprising, given that four of these tumors have LOH at 11q13.

The majority of mutations are deletions that were predicted to result in protein truncation. Missense mutations do not truncate the protein product, yet because proteins have intricate tertiary structure dependent upon interaction between amino acids in the polypeptide chain, the type of amino acid substitution that occurs as a result of the mutation is critical in determining the effect on the protein function. All of the missense mutations reported in this study resulted in nonconservative amino acid substitutions. The change of uncharged polar to positively charged (Q141R, S145R) and positively charged to nonpolar amino acids (R330P) can have a potentially significant effect on the function of the protein. The functional significance of these mutations will be evident once we have a better understanding of wild-type MENIN function.

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REFERENCES

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