Localization of the Multiple Endocrine Neoplasia Type I (MENI) Gene Based on Tumor Loss of Heterozygosity Analysis

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Abstract

Multiple endocrine neoplasia type I (MENI) is an inherited syndrome that results in parathyroid, anterior pituitary, and pancreatic and duodenal endocrine tumors as well as foregut carcinoids in affected patients. The gene responsible for the disease has been linked to chromosome 11q13. We analyzed loss of heterozygosity (LOH) in 188 tumors from 81 patients in an attempt to further define the location of the MENI gene. Both tumors from MEN1 patients and corresponding sporadic tumors were analyzed. Tumor types included parathyroid, gastrinoma, pancreatic endocrine, pituitary, and lung carcinoid. Six tumors (three MEN1 and three sporadic tumors) were identified that provided important LOH boundaries. Four tumors (two parathyroid tumors, one gastrinoma, and one lung carcinoid tumor) showed allelic loss that placed the MENI gene distal to marker PGM. Two tumors (one gastrinoma and one parathyroid tumor) showed an LOH boundary that placed the gene proximal to D11S449, one of which further moved the telomeric boundary to D11S4936. Taken together, the present data suggest that the MENI gene lies between PGM and D11S4936, a region of approximately 300 kb on chromosome 11q13.

Introduction

Identification of the gene responsible for MEN12 is an important goal of the neuroendocrine tumor research community. Characterization of the biological function of the gene will likely yield new insights into the etiology and pathogenesis of these tumors. Additionally, the gene may serve as a future diagnostic or therapeutic target in the management of patients with inherited or sporadic neuroendocrine neoplasms.

The MEN1 gene has been linked to chromosome 11q13 (1–4). High rates of allelic loss in this region have been observed in both tumors from MEN1 patients and from their sporadic counterparts (5–12). In an attempt to narrow the MEN1 gene interval, we analyzed 188 tumors for LOH on 11q13. Both MEN1-associated tumors and sporadically arising tumors were included in the study. Analysis of tumor LOH was performed to define a minimal interval for the location of the MEN1 gene.

Materials and Methods

Archival paraffin-embedded and freshly frozen tumor samples were collected from the files of the Laboratory of Pathology, National Cancer Institute. Tumor material was derived from patients seen at the NIH. Tumor samples included 149 MEN1 tumors from 42 patients and 39 sporadic tumors from 39 patients. Eighty-four parathyroid tumors (80 MEN1 and 4 sporadic tumors), 55 gastrinomas (36 MEN1 and 19 sporadic gastrinomas), 43 islet cell tumors (27 MEN1 and 16 sporadic tumors), and 3 MEN1 pituitary and 3 MEN1 lung carcinoids were studied.

Tissue microdissection was performed as described previously (12, 13) and was recently performed by laser capture microdissection (14). Tumor samples and normal cells were dissected from tissue sections, amplified by PCR, and analyzed by autoradiography. LOH was determined visually, based on complete or near complete loss of an allele. All samples were run in duplicate, and the six critical tumors that provided LOH boundaries were analyzed a minimum of two times.


Results and Discussion

Larsson and coworkers were instrumental in initially identifying chromosome 11q13 as the location of the gene responsible for MEN1, and two groups recently reported recombination analyses that substantially narrowed the candidate region (17, 18). The study by the European Consortium on MEN1 represents the smallest meiotic MEN1 interval to date and localizes the gene to an approximately 2-Mb region (17).

The reliability of LOH analysis in further localizing the MEN1 gene remains an open question. Previously published reports of neuroendocrine tumor LOH are conflicting, making it difficult to confidently assign a location of the MEN1 gene based upon allelic loss (9, 10, 19–21). The recent report by the European Consortium on MEN1 questioned the reliability of tumor LOH in localizing the gene, primarily due to the discontinuous patterns of allelic loss that have been observed (17). Clearly, a tumor LOH boundary that provides an inaccurate interval will significantly damage efforts to locate the MEN1 gene, thus tumor studies must be performed and interpreted with caution (12).

Certainly, LOH analysis of MEN1-related tumors is a difficult task. The combination of normal cell contamination (stromal, endothelial, and normal epithelial), multiple independently arising tumor clones within a single parathyroid gland, DNA recovered from archival formalin-fixed paraffin-embedded tissue, and PCR analysis must be carefully considered. These factors are compounded when newly developed PCR-based microsatellite markers are used that have not been tested extensively on patient tissue samples. Additionally, even with careful analysis of tumors, one must always be cautious of the background LOH that can occur in...
Localization of the MEN1 Gene by LOH

Fig. 1. Schematic diagram of chromosome 11q13 illustrating six tumors that provide LOH boundaries for localization of the MEN1 gene. ◻, allelic retention; □, LOH at informative polymorphic markers. Tumors 1–4, the MEN1 gene is located distal to marker PYGM; Tumor 5, the gene is centromeric to D11S4936; Tumor 6, the gene is centromeric to marker D11S449. The region between PYGM and D11S4936 is approximately 300 kb.

Fig. 2. Schematic diagram of chromosome 11q13. Insets, denaturing electrophoresis gel analysis of polymorphic markers for tumors 4 and 5. N, normal DNA; T, tumor DNA. Tumor 4 shows allelic retention at markers D11S956, D11S1883, and INT2, and shows allelic loss at markers D11S1783, D11S4939, D11S4933, and D11S4908. NT2 shows retention, indicating that the allelic loss does not involve the entire long arm of chromosome 11. Tumor 5 shows LOH at markers D11S956, D11S480, D11S599, and D11S4939 and shows allelic retention at markers D11S4936, D11S449, D11S4933, and D11S2072 showing retention.

The reliability of the present approach is reflected in the frequency of wild-type LOH that was detected in the study. We were able to determine the disease allele in nine MEN1 families by analysis of constitutional DNA from multiple affected family members. Thirty-six tumors from members of these families were analyzed for LOH,

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and 33 of these tumors showed allelic loss on chromosome 11q13. All tumors showed loss of the wild-type allele at every marker tested where LOH was present (as an example, see Fig. 3). Loss of the disease allele was never observed, supporting the notion that neuroendocrine tumor LOH analysis is a robust approach and has utility in defining boundaries for the location of the MENI gene.

There are several caveats we considered when interpreting the present data. Of primary concern is artifactual assignment of allelic retention at a given marker in a tumor with LOH, thereby falsely excluding a portion of the candidate interval. This is particularly problematic with PCR-based analysis. For example, in our experience, if tumor samples contain substantial contaminant normal cells (>10%), then one is forced to consider LOH present based on allelic imbalance. However, PCR primer sets often differentially amplify the contaminant normal allele, making interpretation of LOH difficult and potentially misleading. This difficulty is compounded when allele stutter bands and overlapping alleles are encountered. Careful microdissection of tumors and avoidance of normal endothelial cells when dissecting parathyroid lesions proved valuable to us in the firm assignment of LOH (12).

A secondary danger is artifactual assignment of LOH based on allelic dropout. This is a particular problem when DNA from formalin-fixed paraffin-embedded material is being analyzed. The PCR signal obtained at a polymorphic locus often shows less amplification of the larger allele due to the poor quality of the DNA. If the alleles in a given patient differ substantially in size, the effect can be pronounced and can mimic LOH even when a deletion is not present. Recovery of normal cell DNA from tissue sections is an important control to avoid this problem.

LOH analysis of parathyroid tumors can be problematic due to the presence of multiple independently arising tumor clones with different allelic loss profiles within a single gland (12). Homogenization of whole tumor specimens combines the separate clones and makes interpretation of LOH results difficult at markers where separate tumor clones do not share the same LOH pattern.

However, we believe that tumor LOH analysis can be reliable. Careful microdissection of samples, duplicate PCR reactions with template dilutions, and comparison of tumor results against normal cells procured from tissue sections are useful. The present study identified six tumors that provided boundaries for the MENI gene. Four separate tumors showed PYGM as a proximal boundary that is unlikely to be due to background LOH. Interestingly, both of the cases that provided telomeric boundaries are sporadically arising tumors. The reliability of sporadic tumors in MENI gene localization is less clear, but the fact that the entire data set, including MENI and sporadic tumors, shows an overlapping and internally consistent region of LOH adds confidence to the conclusion that this is the correct interval. Thus the data suggest that tumor LOH represents inactivation of the identical gene in both MENI and sporadic tumors, as predicted by the Knudson model (26), and point to the region between PYGM and D11S4936 as the most likely interval harboring the MENI gene.

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References


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