Two North American Families with Hereditary Papillary Renal Carcinoma and Identical Novel Mutations in the MET Proto-Oncogene

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ABSTRACT

Hereditary papillary renal carcinoma (HPRC) is a newly recognized inherited disorder characterized by a predisposition to develop multiple bilateral papillary renal tumors. Individuals affected with HPRC have been shown to have germ-line mutations in the tyrosine kinase domain of the MET proto-oncogene. We identified a novel mutation in exon 16 of the MET gene in two large North American HPRC families. The H1112R MET mutation segregated with the disease, was not present in 320 normal chromosomes, and caused malignant transformation of NIH 3T3 cells. By examining individuals with the H1112R mutation, we determined the age-dependent penetrance of this mutation and identified additional nonrenal malignancies that occurred in mutation carriers. Affected members of the two families shared the same haplotype within and immediately distal to the MET gene, suggesting a founder effect. The identification of the H1112R mutation will facilitate predictive testing in HPRC and guide future studies of the MET gene in human neoplasia.

INTRODUCTION

HPRC is a recently recognized form of inherited kidney cancer characterized by a predisposition to develop multiple bilateral papillary renal tumors (1-4). Previously, we located the HPRC gene at chromosome 7q31.1-34 by linkage analysis and demonstrated germ-line mutations in the tyrosine kinase domain of the MET proto-oncogene in affected members of four of seven HPRC families (3, 4). Mutations in the MET gene were also detected in a subset of sporadic papillary renal carcinomas. Two mutations in the MET gene were located in codons that are homologous to codons in c-kit and the RET proto-oncogene that are targets of naturally occurring disease-causing mutations.

The results suggested that missense mutations located in the tyrosine kinase domain of the MET proto-oncogene led to constitutive activation of the MET protein and a predisposition to develop papillary renal carcinomas. This concept was supported by studies of transfection of mutant mouse MET genes into NIH 3T3 cells (5). Transfection of mouse MET genes bearing missense mutations identified in HPRC patients produced malignant transformation of NIH 3T3 cells. Constitutive phosphorylation of MET proteins was demonstrated in mouse cells transfected with mutant MET genes.

In this report, we describe a novel missense mutation in exon 16 of the MET proto-oncogene in two large North American HPRC families (families 150 and 160). The mutation is identical in both families; affected members of the two families shared the same haplotype within and immediately distal to the MET gene, suggesting a common ancestor (founder effect). We describe the penetrance of the mutation based on molecular detection of mutation carriers and renal tumor detection by CT. We also examine the involvement of the H1112R MET mutation in the development of other malignancies.

MATERIALS AND METHODS

Subjects. The study was carried out in HPRC families 150 and 160 (1-4). Family members were examined at the Clinical Center of the NIH or at participating institutions after informed consent was obtained. Samples from patients examined at the Albert Ludwig University Freiburg were also tested for MET mutations. This project was approved by the Clinical Research Subpanel of the National Cancer Institute. The evaluation consisted of a history and a physical examination, CT of the abdomen (with and without contrast material), an ultrasound examination of the kidney, and, in selected patients, magnetic resonance imaging examination of the kidney. All subjects had a urinalysis and measurement of serum creatinine, chemistries, and a complete blood count.

An individual was considered affected if he or she had multiple papillary renal carcinomas. The diagnosis of papillary renal carcinoma on family members was made after a review of death certificates, medical records, pathology, and autopsy reports. For asymptomatic members of HPRC families, affection status was determined by CT examination of the kidneys. Asymptomatic individuals free of renal tumors by CT examinations were classified as not affected. An asymptomatic member of an HPRC family was considered affected when the patient was shown to have one or more solid renal tumors with a diameter of 10 mm or greater detected by radiological examinations. All CT films were examined by one radiologist (P.C.) with experience in evaluating the results of CT examinations in inherited renal cancers (6).

Mutation Analysis of the MET Gene. We scanned exons 2, 4, 7, and 9-21 of the MET proto-oncogene for mutations in one affected member of HPRC families 150 and 160 by SSCP (3, 7). We sequenced exons 4, 7, and 10-21 in one affected member of HPRC families 150 and 160. Because the novel nt change in exon 16 identified in affected members of families 150 and 160 was detected by direct sequencing (not by SSCP), exon 16 was sequenced in all family members and in 160 normal individuals.

Nucleotides were numbered according to the scheme in GenBank Accession number J02958, with nt 194 in the cdNA in Ref. 8 given the number 1. Amino acids were numbered as in Ref. 8. Exon-intron boundaries were determined, and intronic primers were designed for SSCP analysis (7); SSCP was performed as described previously (9). PCR conditions and primer sequences are available from the authors. PCR products from samples that showed an aberrant SSCP shift were sequenced as described previously (10).

Haplotype Analysis. Microsatellite analysis was performed as described previously (11) using chromosome 7 tetranucleotide repeat polymorphic markers that were purchased from Research Genetics or were gifts from Dr. Stephen Scherer (Hospital for Sick Children, Toronto, Canada) and Dr. Jeffrey Murray (Cooperative Human Linkage Center, Iowa City, IA). Polymorphisms in the MET gene were identified in exons 20 and 21 by direct sequencing. Haplotypes were constructed based on these polymorphisms.
Penetrance. The risk of developing disease among carriers of the H1112R MET mutation (penetrance) was estimated from Kaplan-Meier curves constructed using the computer program (version Sep/26/84) of Thomas et al. (12). Ages were determined at the time of study for unaffected mutation carriers and individuals who did not carry the mutation. Ages of affected carriers were determined at the time of diagnosis or death. Ascertainment of affection status was determined by CT scan. Binomial 95% confidence intervals were calculated at each point on the curve. All family members older than 20 years who were H1112R MET mutation carriers were examined by CT with the exception of a single woman (patient 3757; age, 85 years) who was omitted from the analysis; an examination of patient 3757 by ultrasound did not show renal tumors.

Restriction Endonuclease-based Mutation Detection Assay. A PCR-based restriction endonuclease assay was used to confirm the MET mutation identified in families 150 and 160. Intronic primers were designed to amplify the region of exon 16 containing the mutation (forward primer, 5'-ATTAAAT-GTTACCGAGTCTAAC; and reverse primer, 5'-ATGTGTTAATAAAAAAT-GCCACTTAC). PCR products were purified by the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and digested with Mbol (New England Biolabs, Beverly, MA) according to the manufacturer’s protocol. Restriction fragments were separated on a 4–20% gradient polyacrylamide gel (Novex, San Diego, CA) in 1 x Tris-borate EDTA buffer at 200 V for 1 h. In wild-type DNA, fragments of 81 and 68 bp were generated; the H1112R mutation destroyed the restriction site, leading to a mutant allele of 149 bp.

NIH 3T3 Transformation Assay. NIH 3T3 cells (CRL 1658) were obtained from the American Type Culture Collection (Rockville, MD) and cultured in DMEM with 10% calf serum (Life Technologies, Inc., Gaithersburg, MD). The pMBI expression vector containing wild-type mouse MET cDNA (pMBI1) was used for mutation construction (5). To construct the MET mutants, the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) was used with pMBI1 as the template. Mutations were verified by sequencing both strands of DNA in the region of interest.

Transfections were performed as described previously with a plasmid containing the mutant or wild-type MET construct, a plasmid conferring resistance to G418 (pSV2neo), and LipofectAMINE (Life Technologies, Inc.). Focus formation assays were performed as described previously (5).

In Vivo Tumorigenicity Assay. Pools of G418-resistant NIH 3T3 cells expressing the mutant MET protein were generated as described above and in Ref. 5. Cells (5 x 10^5) were injected s.c. into 4-week-old female athymic nude mice. Mice were examined weekly, and the number and size of tumors were recorded.

RESULTS

HPRC families 150 and 160 have been described previously (1–4). There are 9 individuals affected with HPRC in family 150 and 13 individuals affected with HPRC in family 160. A total of 31 members of family 160 and 15 members of family 150 were examined by CT for the presence of renal tumors. Occult asymptomatic renal tumors were found in three members of family 150 and four members of family 160.

MET Mutation Detection. By direct sequencing of the MET proto-oncogene, we identified an A to G change at nt 3529 that changed a histidine at position 1112 to an arginine (H1112R) in affected members of both families 150 and 160 (Fig. 1A). This missense mutation did not produce a change in amino acid class, because histidine and arginine are amino acids with positively charged side chains. The H1112R mutation was confirmed by a PCR-based restriction endonuclease assay (data not shown). The H1112R mutation was located adjacent to the glycine-rich loop of the tyrosine kinase domain (Fig. 2B).

To determine whether H1112R was likely to be a disease-producing mutation, we tested whether the mutation segregated with the disease and was present in the normal population and evaluated the function of the mutant protein. The H1112R MET mutation segregated with the disease in HPRC families 150 and 160 (Fig. 1B). No individual without the H1112R MET mutation had a renal tumor; overall, 22 of 32 family members with the H1112R mutation had renal tumors; 12 of 13 individuals over 50 years of age who carried the H1112R mutation had renal tumors. The H1112R MET mutation was not detected by direct sequencing in 160 normal individuals (320 chromosomes). These results suggested that the H1112R mutation was not a rare polymorphism.

An identical germ-line H1112R MET mutation was also detected in a German patient (patient 5976) with bilateral papillary renal carcinoma without a family history of renal malignancy.

NIH 3T3 Transformation Assay. Previously, we demonstrated that transfection of mouse MET DNA genes containing the mutations identified in HPRC patients produced malignant transformation of NIH 3T3 cells (5). To evaluate the transforming potential of the H1112R mutation, the H1112R mutation was introduced into a vector (pMBI1) containing the mouse MET proto-oncogene with a long terminal repeat promoter. The biological properties of this construct were evaluated on NIH 3T3 cells. The H1112R mutation produced an average of 59 foci/μg DNA in two separate experiments and produced tumors at the site of inoculation in 10 of 10 nude mice in 2–3 weeks (Fig. 1C). An average of three foci were produced by the transfection of NIH 3T3 cells with wild-type mouse MET, and no tumors were detected at 3 weeks after inoculation.

Penetrance. The availability of 23 individuals with the identical mutation in the MET proto-oncogene examined by CT at a single
screened at the NIH 1 year before his death, and no renal tumors were detected by CT. Pathological examination of the kidney did not detect precursor lesions in the kidney of this H1112R mutation carrier. No detectable renal alterations were produced in this patient by the MET H1112R mutation by 22 years of age.

Tests for Founder Effect. The presence of the identical mutation in the MET proto-oncogene in families 150 and 160 raised the possibility of a founder effect. Family 160 is a Canadian family; family 150 is from the United States, but family members lived in Canada during the 19th century. Haplotype analysis showed that affected members of these two families share alleles at two polymorphic sites within the MET gene (nt 4106 and nt 4340) and two tetranucleotide repeat polymorphic markers (D7S2847 and D7S1835) immediately distal to MET (data not shown). These results suggest that a founder effect is responsible for HPRC in these two families. Because the German family contained only one affected individual, we were unable to determine the haplotype surrounding the H1112R MET mutation in patient 5976.

Other Tumors in H1112R Mutation Carriers. To evaluate phenotypic effects of the H1112R mutation in MET, we tabulated all malignancies known to be present in members of families 150 and 160 in individuals who were and were not mutation carriers (Table 1). Tumors found in carriers of the H1112R mutation in addition to papillary renal carcinomas included a biliary tract carcinoma (Klatskin tumor), carcinomas of the pancreas, malignant melanoma, and breast carcinoma.

DISCUSSION

We found a germ-line missense mutation (H1112R) in the tyrosine kinase domain of the MET proto-oncogene in two large North Amer-
MET MUTATIONS IN TWO FAMILIES WITH HPRC

American families with HPRC. Our results suggest that the H1112R was a disease-producing mutation. The H1112R mutation segregated with disease and was not found in a panel of 320 normal chromosomes. Functional studies demonstrated that the mouse MET gene with the H1112R mutation produced malignant transformation of NIH 3T3 cells.

Previously, we identified missense mutations in exons 17, 18, and 19 of the MET proto-oncogene. The H1112R mutation is the first mutation to be identified in exon 16. The previously described mutations in MET were located in the COOH-terminal lobe of the tyrosine kinase domain and were clustered in the activation loop (5). The H1112R mutation is located in the NH2-terminal lobe of the tyrosine kinase domain adjacent to the highly conserved glycine-rich region (GXGXXGXV). This region of the tyrosine kinase domain is involved with the binding of ATP and maintaining the proper orientation of the NH2- and COOH-terminal lobes of the tyrosine kinase domain (13).

With the identification of the H1112R MET mutation, six of the seven families studied in our laboratory characterized by multiple bilateral papillary renal carcinomas in two generations have been shown to have germ-line mutations in the MET proto-oncogene. The results of penetrance studies indicate that the MET H1112R mutation produces a neoplastic disease that may be detected as early as 30 years of age but is generally detected after 50 years of age. Although the data suggest that eventually all carriers of the H1112R mutation will develop renal neoplasms, this conclusion must be viewed with caution because of the limited number of patients studied and the wide confidence limits.

We observed considerable variation in the number of renal tumors in carriers of the H1112R MET mutation. In part, this may be a reflection of the age at which patients were examined. Patients 20–35 years of age showed 1–2 renal neoplasms by CT. However, even among H1112R individuals of the same age, there was a considerable variation in the number of renal neoplasms (range, 0 to >100). The disparity in the renal tumor number in individuals of the same age who are mutation carriers raises the possibility of other genes that may modify the expression of the mutation in the MET gene.

The presence of additional neoplasms in carriers at the H1112R mutation raises the possibility that the H1112R mutation may play a role in the pathogenesis of carcinomas of the pancreas and biliary tract. Additional studies of these neoplasms are needed to evaluate this possibility.

ACKNOWLEDGMENTS

We thank Charles Riggs of Computer and Statistical Services, Data Management Services, Inc. for statistical analysis of the penetrance data.

REFERENCES

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