A Novel Missense Mutation of the MEN1 Gene in a Multiple Endocrine Neoplasia Type 1 Patient Associated with Carcinoid Syndrome

Chizuko Ukita, Makiko Yamaguchi, Toru Tanaka, Hirofumi Shigeta and Mitsushige Nishikawa*

Abstract

We report a multiple endocrine neoplasia type 1 (MEN1) patient associated with carcinoid syndrome. A 50-year-old woman had parathyroid hyperplasia with primary hyperparathyroidism, a pancreatic tumor and carcinoid tumors in the liver and duodenum. The primary lesion of the carcinoid was probably the bronchus. Direct sequencing analysis revealed a novel missense mutation at codon 342 in exon 7 causing an amino acid change from alanine to proline (A342P) of the MEN1 gene. Loss of heterozygosity (LOH) was also detected in the resected parathyroid tissue. This mutation appeared to play an important role in the tumorigenesis of the endocrine tissues in the present case.

Key words: MEN1, mutation, carcinoid, hyperparathyroidism, menin, loss of heterozygosity, tumorigenesis

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder, the typical clinical spectrum of which includes tumors of parathyroid, endocrine pancreas and pituitary. Other less common lesions are adenomas of thyroid and adrenal glands, carcinoid tumors, and lipomas (1-3). The association of carcinoids with MEN1 has been reported since 1953 (4), and carcinoids are found in 16-20% of patients with MEN1 (2, 3).

The MEN1 gene locus was mapped to chromosome 11q13 (5), and was cloned in 1997 (6). The MEN1 gene contains 10 exons and encodes a 610-amino acid protein, denoted menin (6). Although the functional role of menin remains to be elucidated, it has been suggested to suppress tumorigenesis. Allelic loss of heterozygosity (LOH) for 11q13 has been reported in 63–100% of MEN1-associated parathyroid tumors (7). More than 100 germline mutations of MEN1 gene have been identified in MEN1 patients (8-11).

Here, we report an MEN1 patient associated with carcinoid syndrome, who revealed a novel germline missense mutation of the MEN1 gene and LOH in the parathyroid tissue.

Case Report

A 50-year-old woman was referred to our hospital in May 2000 for further examination of hemoptysis and flushing. In 1985, she was diagnosed as having a duodenal ulcer. The following year, chest x-ray taken during the annual check-up indicated an abnormal shadow. The definite diagnosis of the lesion was unclear in spite of a further evaluation. In 1993, she began to complain of painful attacks of urolithiasis and intermittent history of cough and hemoptysis. In 1999, she experienced an episode of cutaneous flushing on the face, anterior chest and forearm.

On admission, cutaneous flushing on the face was noted. No mass was palpable in her neck. No pathological finding was observed in the chest and the abdomen. Laboratory examination showed elevated serum Ca (12.5 mg/dl) and a decreased P concentration (2.2 mg/dl) with an elevated serum intact parathyroid hormone (PTH) level (120 pg/ml). Serum level of serotonin (1,038 ng/ml) was elevated, as was urinary excretion of 5-hydroxy indole acetic acid (5-HIAA) (54.6 mg/day). Although her serum gastrin level was elevated (650 pg/ml), levels of other pancreatic hormones (insulin: 12 μU/ml, glucagon, somatostatin) were almost normal.
Endocrine studies did not reveal any abnormalities concerning the pituitary (adrenocorticotropic: 31 pg/ml, thyroid-stimulating hormone: 3.25 μU/ml, growth hormone: 3.12 ng/ml, luteinizing hormone: 10.5 mIU/ml, follicle-stimulating hormone: 33.4 mIU/ml, and prolactin: 9.7 ng/ml), thyroid and adrenal glands.

A chest X-ray and computed tomography (CT) of the chest showed a mass with ill-defined margins, 36×40 mm in diameter, in the anterior basal segment of the right lower lobe (S₈). Bronchosopic examination revealed complete obstruction of the right lower lobe bronchus at the origin of the anterior basal branch, and the biopsy specimen revealed class III, but immunohistochemical study was not performed. CT scans of the liver showed multifocal lesions, maximum 50×45 mm in segment 8 (S₈), compatible with metastatic tumor. The tumor cells with a percutaneous needle biopsy of the liver were histologically monotonously similar, having a scant cytoplasm and an oval-shaped nucleus, and partly formed strands (Fig. 1A). Immunohistochemical studies showed positive staining for chromogranin A (Fig. 1B) and synaptophysin (Fig. 1C); the diagnosis of carcinoid tumor was made. Abdominal CT also showed a pancreatic tumor, 10 mm in diameter, in the head of pancreas. In July 2000, she was diagnosed with primary hyperparathyroidism, and underwent total parathyroidectomy. The pathologic diagnosis was hyperplasia of four parathyroid glands. After the surgery, serum Ca, PTH and gastrin returned to normal levels. Upper gastrointestinal endoscopy demonstrated a submucosal tumor, 15 mm in diameter, in the second portion of the duodenum. The tumor was resected in October 2000, and was immunohistochemically positive for chromogranin A; the tumor was also diagnosed as a carcinoid. Magnetic resonance image (MRI) revealed no pituitary lesion.

After the diagnosis of carcinoid syndrome, subcutaneous administration of octreotide at 100 μg/day was begun. The episode of flushing disappeared, the serum level of gastrin
remained within the normal limit, and urinary excretion of 5-HIAA showed a tendency to decrease. She was treated with hepatic arterial embolization in March 2001, and the size of the tumor in S8 was slightly reduced. Subsequently she was treated with bronchial arterial embolization in April 2001, the size of the tumor containing atelectasis was remarkably reduced. She was treated again with hepatic arterial embolization in November 2001, and percutaneous ethanol injection therapy (PEIT) was given for the remaining lesions. In a recent CT examination in 2002, the pancreatic tumor was the same size and shape and no malignant change was suspected. The grandmother of the patient died from duodenal ulcer. The younger sister of the patient suffered from duodenal ulcer from the age of 40, but she has no lesion of the parathyroid or pancreas. Other family history was unremarkable. Unfortunately the immediate family members refused endocrine examinations.

**Mutation analysis of MEN gene by direct DNA sequencing**

Genetic analysis of the patient’s MEN gene was recognized by the ethical committee of our hospital (No.19) and informed consent was obtained from the patient. Genomic DNA was isolated from peripheral leukocytes using a DNA Extraction Kit (Talent srl, Trieste, Italy). The MEN gene exons 2–10 were amplified with a polymerase chain reaction (PCR), as previously described (12). The amplified PCR products were purified and sequenced directly using sequencing primers with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA), and an automated ABI Prizum 310 Genetic Analyzer (Perkin-Elmer).

**LOH analysis in the parathyroid gland**

A hyperplastic parathyroid gland was obtained at the time of surgery. Genomic DNA was isolated from parathyroid tissue with Takara Dexpat (Takara, Osaka, Japan). Allelic loss of the MEN1 gene was assessed by microsatellite analysis using DNA markers on chromosome 11q13, D11S4940. DNA was amplified by PCR with a pair of primers, one of which was labelled with fluorescein (Cy5). The amounts and sizes of the PCR products were determined by electrophoresis, and fluorescence intensity was measured with an automated sequencer (ALF express: Pharmacia, Freiburg, Germany).

**Results**

Direct sequencing of genomic DNA extracted from the leukocytes revealed G-to-C transition at codon 342 in exon 7 (Fig. 2) causing an amino acid change from alanine to proline (A342P) of the MEN1 gene. LOH was examined in the hyperplastic parathyroid gland. Microsatellite polymorphism analysis of D11S4940 showed a reduced fluorescence peak in tissue DNA, compared with that in blood DNA (Fig. 3).

**Discussion**

The typical clinical spectrum of MEN1 includes tumors of parathyroid, endocrine pancreas and pituitary, which occurs in more than 95%, 40–50%, and 30% of MEN1 patients, respectively (1–3). Traditionally, determination of the diagnosis of MEN1 depends on the identification of 2 or more MEN1-related major tumors, as well as a family history of endocrinopathy. Carcinoids are less common in MEN1, and most carcinoids associated with MEN are of foregut origin; bronchus, thymus and duodenum, in contrast to the usual midgut and hindgut origin (13). Bronchial carcinoids associated with MEN are more common in women, and most are benign (13). In the present case, parathyroid hyperplasia with hyperparathyroidism and carcinoid in the liver and
duodenum were pathologically diagnosed. She also had a pancreatic tumor, which was not thought to produce gastrin, since hyperparathyroidism with resultant hypercalcemia may stimulate gastrin release (14) and the serum gastrin concentration returned to normal after parathyroidectomy. Furthermore, as no symptoms due to other excessive hormones were obvious, the pancreatic tumor seems nonfunctional, although no histological data were available. Pituitary MRL showed no pituitary lesion. Pulmonary lesion was not diagnosed histologically this time, but the bronchus was suspected to be the origin of her carcinoid, because the pulmonary lesion was documented to exist in 1986, the doubling time of the tumor size was about four years and because the bronchus is a common lesion of carcinoid associated with MEN1. Based on the above results, she was diagnosed as having MEN1.

Various mutations have been reported in the MEN1 gene to date (8–11), but no genotype-phenotype correlation has been established. A missense mutation E45G was identified in a case of thymic carcinoid in a family of MEN1 (15). On the other hand, MEN1 gene mutations were not identified in 2 cases of MEN1 with gastric carcinoid (16, 17). In the present case associated with carcinoid tumors in the liver, duodenum and possibly bronchus, a germline missense mutation (A342P, exon 7; the codon number is defined based on isoform 2) was identified. This mutation has not been reported to date in The Genome Database (GDB), National Center for Biotechnology Information (NCBI) and Gene Cards (the codon numbers in these data base are based on isoform 1), and have not been observed in normal volunteers. Therefore, A342P is likely to be a MEN1-specific mutation. The germline mutations include frameshift, nonsense, missense and in-frame deletion mutations. The former two result in truncation of menin most likely to lose its function, though the consequence of the latter two mutations are not obvious (6, 8). There is no direct evidence for the role of MEN1 gene mutations in the tumorigenesis of MEN1. Although the functional role of menin remains to be elucidated, recent reports suggest that menin is a nuclear protein (18), interacts with the transcription factor JunD and represses JunD-activated transcription (19). Both nuclear localization signals (NLS) -1 (amino acids 479–497) and NLS-2 (amino acids 588–608) were identified in the C-terminal portion of menin (18). Furthermore, two separate regions of menin, 139–242 and 323–428, separately bind JunD (19). It is therefore probable that this novel mutation of A342P might interfere with the binding of menin with JunD, suggesting that the mutation is likely to affect the functional activity of menin.

LOH on chromosome 11q13 has been reported in 63–100% of MEN1-associated parathyroid tumors (7). A tumor suppressor role for the MEN1 gene has been suggested, based on Knudson’s hypothesis; in most tumors in familial cases, the affected individuals carry a mutant tumor suppressor gene on one allele, and somatic events on the other allele causing a loss of function of the gene, leading to tumorigenesis (20). The high prevalence of heterozygous germline mutations of the MEN1 gene and tissue LOH reported in many studies appears to be compatible with the hypothesis. In the present case, we also showed LOH in the parathyroid gland suggesting that loss of function of menin plays an important role in the tumorigenesis of parathyroid glands as previously reported (21, 22). Germline mutation of the MEN1 gene and tissue LOH in the parathyroid gland provide strong evidence that this was the mutation responsible for MEN1 in the present case. As we did not perform LOH study in the liver or duodenum, it is unclear whether A342P is the mutation responsible for carcinoid or not. Genetic analysis is thus very useful for such a variant case as well as for other typical cases of MEN1.

Of MEN1 patients, 43.5–46% died of causes related to their endocrine tumors, and 22% died of carcinoid (23, 24). Metastatic carcinoids have an average survival of 24 months from the time of diagnosis of hepatic metastasis (25). In 1995, Diaco et al reported that long-term octreotide acetate therapy, when combined with intraarterial chemotherapy and chemoembolization, appeared to be more effective than mechanical tumor embolization alone, intravenous chemotherapy alone, or octreotide therapy alone for control of tumor growth and carcinoid syndrome (26). In the present case, octreotide therapy combined with embolization and PEIT decreased tumor size slightly and improved her symptoms. Octreotide can have a direct antitumor effect as a tumor reportedly disappeared following intraarterial infusion of octreotide (27). Since the intraarterial infusion of octreotide may be a useful therapy for liver metastasis from carcinoid, this procedure may be another choice of treatment for the present patient.

Acknowledgements: The authors are grateful to Dr. Toshihiko Tsukada, Growth Factor Division, National Cancer Center Research Institute, for his valuable advice.

References


