Unusual Clinical and Pathological Presentation of a Neuroendocrine Tumor in a Patient with Multiple Endocrine Neoplasia Type 1

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Abstract. Neuroendocrine tumors develop in various organs in patients with multiple endocrine neoplasia type 1 (Men1). Among those, tumors developed in upper gastrointestinal tract, thymus and bronchus have historically been called “carcinoid tumor”. Occurrence of “carcinoid tumor” in other region is very rare and molecular pathogenesis of such tumors is unknown. We have experienced a patient with Men1 who have developed an “ectopic” retroperitoneal neuroendocrine tumor. Genetic analysis of the MEN1 gene in tumor cells revealed a somatic mutation in exon 9 as well as a germline mutation in exon 10. Allele-specific amplification followed by sequence analysis revealed these two mutations exist on the different allele, indicating both alleles are functionally inactivated. Immunohistochemical staining with an anti-menin antibody revealed that wild-type menin is not expressed in tumor cells. Expression of p27kip1 protein is not observed in tumor cells, either. These results confirmed the inactivation of the MEN1 gene as a genetic cause of an ectopically developed neuroendocrine tumor in a patient with Men1.

Key words: Loss of heterozygosity, Two hit theory, p27, Tumor suppressor

MULTIPLE endocrine neoplasia type 1 (Men1) is a relatively rare autosomal dominantly inherited disorder characterized by hyperplastic and neoplastic disorder of endocrine organs such as parathyroid, anterior pituitary and gastroenteropancreatic endocrine tissues [1]. Most subjects with Men1 carry a heterozygous germline mutation in the MEN1 gene which localizes to chromosome 11q13 and encodes 615 amino-acid*1 nuclear protein menin [2]. The physiological roles of menin remain largely elusive, but there are several evidences that menin interacts directly or indirectly with various cellular proteins and functions as a tumor suppressor [3]. Recent studies have revealed that menin regulates expression of cyclin-dependent kinase inhibitor p18\(^{\text{INK4c}}\) and p27\(^{\text{kip1}}\) [4, 5] and inactivation of rat Men1 gene causes reduction of p27\(^{\text{kip1}}\) expression, which may partly explains molecular mechanism of tumorigenesis due to MEN1 inactivation [6, 7]. Subsequently, homozygous germline mutation of the Cdkn1b, encoding p27\(^{\text{kip1}}\), was identified in the rat with MENX, a recessive MEN-like syndrome [8]. Rats with MENX manifest phenotypic features overlapping both Men1 and MEN2 including parathyroid adenoma, C cell hyperplasia of thyroid and pheochro-

1 Alternative splicing at exon 2 of the MEN1 gene produces two isoforms of menin, 610 and 615 amino acids in length, respectively. Numbering of amino acid and nucleotide in this manuscript is based on that for 615 amino acid protein.
mocytoma/paraganglioma [9]. Germline CDKN1B mutations have also been identified in a limited subset of patients with MEN1 who do not carry MEN1 mutation [8, 10].

Less frequent manifestations include adrenal cortex adenoma, various cutaneous tumors and neuroendocrine tumors in thymus, bronchus and stomach/duodenum [11-15]. Among those, thymic neuroendocrine tumor has a high malignant potency accompanying a recurrence and distant metastasis [16, 17]. Association of MEN1 with neuroendocrine tumor of other organs has not been recognized. As far as we are aware, there has been only one article reporting MEN1 case with “carcinoid tumor” occurred outside of common organs (e.g. thymus, bronchus, stomach and duodenum) [18]. Morgello et al. reported a 63-year-old woman who had presented insulin overproduction from an ovarian carcinoid. Autopsy of this patient also revealed a solitary parathyroid adenoma and a pituitary hyperplasia, and those findings made authors consider the possibility of MEN1. However, since solitary parathyroid adenoma is not a common feature of MEN1, and considering the age of the patient at autopsy, diagnosis of MEN1 in this patient is doubtful.

We have experienced a patient with MEN1 who had developed a retroperitoneal neuroendocrine tumor which we initially suspected as being paraganglioma occurred from the organ of Zuckerkandl. We analyzed involvement of MEN1 gene in the development of this tumor and also examined expression of menin and p27Kip1 protein in tumor cells.

**Patient and Methods**

**Case Report**

The patient was a 63-year-old Japanese woman. In 1994, at the age of 50, she visited another hospital because of persistent headache and amenorrhea, and was found to have a non-functioning pituitary tumor. During hospital stay, elevated levels of serum calcium and parathyroid hormone were also found. With the clinical diagnosis of MEN1, she had had transphenoidal surgery for a pituitary tumor and total parathyroidectomy with autotransplantation for primary hyperparathyroidism. Since then, her pituitary- and parathyroid functions have been maintained normal. Later, she had had a genetic testing and a heterozygous frameshift mutation in exon 10 of the MEN1 gene (c.1561_1562insC*2) had been identified [19]. This mutation has been described as c.1546_1547insC in previous studies [2].

Fig. 1. CT image of the abdomen showing a tumor with the size of 65 mm x 50 mm (arrows).

*2 This mutation has been described as c.1546_1547insC in previous studies [2].
Results

Pathological Analysis

Pathological examination of the resected tumor revealed that acidophilic tumor cells appeared in well-defined nests and were separated by fibrous septa, showing a characteristic pattern of “Zellballen” structure, a hallmark of paraganglioma (Fig. 2A). Thus we initially thought that this tumor as a paraganglioma occurred from paraaortic sympathetic ganglia. By immunostaining, tumor cells were strongly positive for synaptophysin, but negative for chromogranin A (Fig. 2, B and C). The latter was an atypical finding for paraganglioma. Additional immunostaining for cytokeratin showed strong positive signal, albeit not all cells were stained, suggesting this tumor as epithelial origin (Fig. 2D). S-100 was positive only in part of sustentacular cells. Based on these results, tumor was pathologically
diagnosed as a well-differentiated neuroendocrine carcinoma. Preoperative measurement of catecholamines and serotonin was not done because possibility of paraganglioma or neuroendocrine tumor was not considered before surgery, but abnormal elevation or fluctuation of blood pressure and blood glucose was not seen before, during or after operation. Symptoms accompanied by so-called carcinoid syndrome such as watery diarrhea and flushing, cyanosis were not seen, either.

**Genetic Analysis**

To examine if alteration of the *MEN1* gene is involved in the development of retroperitoneal neuroendocrine tumor in our patient, DNA was isolated from peripheral leukocytes and microdissected tumor tissue. This study was reviewed and approved by the Ethics Review Committee for Human Gene Analysis Research of Shinshu University. Entire coding region and adjacent exon-intron boundaries were amplified and sequenced. As previously examined, heterozygous 1 base insertion (c.1561_1562insC) was identified in exon 10 in DNA isolated from both leukocytes and tumor cells (Fig. 3A). This mutation causes a frameshift at amino acid codon 521. Additionally, heterozygous 1 base deletion (c.1232delA) was found in DNA obtained from tumor cells but not in leukocyte DNA (Fig. 3B). This deletion, which causes a frame-shift at amino acid codon 411 and premature termination of the protein 37 amino acids downstream, has neither been reported nor registered in Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php).

If this somatic mutation exists on the wild-type allele, both alleles in tumor cells are expected to be inactive, which is consistent with Knudson’s “two-hit theory” for development of the tumor [21]. Therefore, we next examined allelic relation of two mutations, germline mutation in exon 10 and somatic mutation in exon 9, by allele-specific amplification. As depicted in Fig. 3C, allele-specific primers were prepared according to the sequence of exon 9 (WF for wild-type sequence and MF for mutated sequence), and DNA obtained from tumor cells was amplified with either WF or MF as a forward primer and 10R as a common reverse primer. Nucleotide sequence of exon 10 in each amplicon was examined. When WF and 10R was used for PCR, nucleotide sequence of amplicon was unable to determine probably due to nonspecific reaction of the forward primer and possible contamination of non-tumor cells in tissue sample. However, when MF and 10R was used, amplified fragment contained only wild-type sequence (Fig. 3D), suggesting somatic mutation in exon 9 exists on the allele without germline mutation. A pair of MF and 10R did not amplify DNA from leukocytes.

**Immunohistological Analysis of Expression of Menin and p27kip1**

Results of genetic analysis described above indicate that intact menin protein may not be expressed in tumor cells. Thus we next examined expression of menin by immunostaining. As shown in Fig. 4A, expression of menin was not observed in tumor cells of our patient, concordant with results of genetic analysis. In contrast, strong nuclear expression of menin was seen in control neuroendocrine tumor which was obtained from a patient without *MEN1* (Fig. 4C).

Involvement of p27kip1 protein in the development of *MEN1* has been recently reported and is receiving a scientific attention [22]. Since menin regulates expression of p27kip1 [4, 5] and inactivation of the Men1 gene causes reduction of p27kip1 expression in rats [6, 7], we also examined expression of p27kip1 in tumor cells obtained from our patient. As expected, the tumor of our patient did not express p27kip1, while strong expression of p27kip1 was observed in the nucleus of control tumor tissue (Fig. 4B, D).

**Discussion**

*MEN1* gene product, menin, is a 615 amino-acid nuclear protein and is widely expressed including non-endocrine organs [23-25]. Menin is believed to function as a tumor suppressor through interaction with various cellular proteins [3], thus organ-specific tumor formation in *MEN1* suggests tissue-specific interaction of menin with other cellular factors or organ-specific post-translational modification of menin. In general, tumor suppressor gene retains normal function when at least one of two alleles is kept intact; both alleles of the relevant gene are functionally inactivated in tumor cells. In *MEN1*-related tumors such as parathyroid hyperplasia, pituitary adenoma and gastroenteropancreatic endocrine tumor, wild-type allele of the *MEN1* gene is somatically deleted in most cases [26-29]. Deletion of the *MEN1* gene locus together with somatic muta-
Fig. 3. Genetic analysis of the tumor and peripheral leukocytes. A, Germline mutation in exon 10 (c.1561_1562insC). This mutation was seen in both tumor cells and peripheral leukocytes. B, Somatic mutation in exon 9 (c.1232delA). This mutation was seen in tumor cells, but not in peripheral leukocytes. C, Schematic representation of the allele-specific amplification. WF and MF represent wild-type allele-specific forward primer and mutant allele-specific forward primer, respectively. Open circle and closed circle represent position of germline mutation and somatic mutation, respectively. D, Nucleotide sequence of the DNA amplified from tumor cells using MF and 10R. Only the allele without germline mutation was amplified.
report on “ectopic” neuroendocrine tumor in a patient with possible MEN1 [18]. Therefore, we examined if MEN1 gene had involved in tumor development of our patient, and found that both MEN1 alleles are functionally lost by germline- and somatic mutations. To date, more than 450 different germline MEN1 mutations have been identified in patients with MEN1 [2]. A germline frame-shift mutation identified in our patient, c.1561_1562insC, is expected to produce a truncated protein which lacks nuclear localization signal. Inability of this mutant protein to localize in nucleus has been shown in vitro [25]. Somatic mutation we identified is also expected to produce a truncated protein which lacks nuclear localization signal. Loss of p27kip1 expression further supported lack of functioning menin protein in tumor cells of our patient.

Origin of the neuroendocrine tumor of our patient is unknown since the tumor was unable to completely resect. It is possible that this tumor had originated from left kidney, although CT scan image seems that tumor and kidney are separated. Alternatively, tumor had developed from aberrant neuroendocrine tissue in

Fig. 4. Immunostaining of the tumor. A and B are retroperitoneal neuroendocrine tumor of the present case and C and D are tumor of the control patient who is not affected with MEN1. Antibody used for each immunostaining is; A and C, anti-menin antibody; B and D, anti-p27 antibody. Original magnification, x100.

A 

B 

C 

D 

值得一提的另一条染色体在某些程度上也观察到在 sporadic parathyroid- and gastroenteropancreatic tumors [30-35]. On the other hand, loss of heterozygosity in the MEN1 gene locus is less frequently observed in thymic carcinoid [16, 36]. These observations suggest that normal allele of the MEN1 gene in thymic carcinoid may be inactivated by other mechanisms such as point mutation (as is seen in our case) or transcriptional inhibition by promoter methylation. Alternatively, other genetic factor(s) other than the MEN1 gene might be involved in tumor development.

In the present study, we reported a retroperitoneal neuroendocrine tumor found in a patient with MEN1. Neuroendocrine tumor can develop in a wide range of organs. Lung and gastrointestinal tract is the most commonly involved organ but other organs such as thymus, testis and ovary are also involved [37]. Renal carcinoid tumors have been reported as a rare occasion [38, 39]. Although neuroendocrine tumor is not uncommon in MEN1, those are exclusively localized in anterior pituitary, thymus, bronchus, and stomach/duodenum. As far as we are aware, there has been only one
Canavase et al. examined \( p27^{kip1} \) expression in more than 100 endocrine tumors of the pancreas and gastrointestinal tract [40]. They found that \( p27^{kip1} \) expression is high in most benign or well differentiated tumors but low in poorly differentiated tumors. It is unknown if some of those tumors had any abnormalities in \( MEN1 \) gene function.

There is no known effective treatment for \( MEN1 \)-related neuroendocrine tumor of thymus. Meanwhile, gastric/duodenal neuroendocrine tumors in \( MEN1 \) patients show relatively indolent clinical course. Retroperitoneal neuroendocrine tumor in our patient showed some malignant characteristics as it had invaded adjacent tissues. As of March 2009, three years after surgery, there are no clinical findings suggesting distant metastasis or massive growth of the primary tumor. Intensive and continuous monitoring of the tumor is scheduled for our patient.

In conclusion, we have experienced a patient with \( MEN1 \) who had developed a neuroendocrine tumor in the retroperitoneal region. Genetic- and immunological examination revealed that expression of normal menin in the tumor was lost, demonstrating involvement of the \( MEN1 \) gene in tumor development.

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