Primary Hyperparathyroidism Associated with Aldosterone-Producing Adrenocortical Adenoma and Breast Cancer: Relation to *MEN1* Gene

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Abstract

A rare case of primary hyperparathyroidism associated with primary aldosteronism and breast cancer is reported. A 44-year-old woman was admitted to our hospital to undergo surgical removal of breast cancer. She had hypertension with low serum potassium, and slightly but significantly elevated serum calcium levels. Further studies demonstrated an enlarged left superior parathyroid gland and a left aldosterone-producing adrenocortical adenoma. Blood pressure was controlled with spironolactone and nifedipine, and left mastectomy was done for breast cancer. The pathological diagnosis was scirrhous breast carcinoma. Although the postoperative course was uneventful, her serum calcium gradually and progressively rose to higher levels. Left superior parathyroidectomy and left adrenalectomy were then performed simultaneously. The pathological diagnoses of the resected parathyroid gland and adrenal gland were parathyroid chief cell adenoma and adrenocortical adenoma with hyperplasia of zona glomerulosa, respectively. To clarify if the occurrence of these tumors may be related to *MEN1* gene mutations, we analyzed *MEN1* gene in this patient, and found a loss of heterozygosity of the *MEN1* locus in the parathyroid adenoma and breast cancer. Thus, we conclude that an alteration of the *MEN1* gene and/or another tumor suppressor gene located at the *MEN1* locus on chromosome 11q13 may be responsible for the development of parathyroid adenoma and breast cancer in our patient suggesting that the clinical spectrum of *MEN1* might include breast cancer. In addition, serum calcium should be interpreted with caution in primary aldosteronism, because hypercalcemia may be masked in the presence of aldosterone excess.

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Key words: multiple endocrine neoplasia type 1 (MEN1), *MEN1* gene, loss of heterozygosity, breast cancer, primary hyperparathyroidism, primary aldosteronism

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited disorder characterized by the presence of hyperplasia or adenoma of the parathyroid gland, and tumors of the endocrine pancreas, and anterior pituitary (1). The responsible gene, *MEN1*, was mapped to chromosome 11q13 (2), and recently identified (3, 4). Since loss of heterozygosity (LOH) of the *MEN1* locus is frequently observed in MEN1-related tumors, the *MEN1* gene has been considered to be a tumor suppressor gene (2, 5–7). Allelic losses of DNA markers on chromosome 11q13, centered on the *MEN1* critical region, in a substantial number of sporadically occurring parathyroid adenomas (6, 7), may further support the notion that the *MEN1* tumor suppressor gene may also be the target of somatic inactivating lesions in sporadic endocrine tumors.

Although the parathyroid, pancreas and pituitary are most frequently affected in MEN1, it is well known that tumors may arise in a variety of organs apparently unrelated to MEN in this pathologic state. One of the rare associations with MEN1 is primary aldosteronism. Here, we report a patient with an unusual combination of primary hyperparathyroidism, primary aldosteronism and breast cancer. To determine whether the development of these tumors may be

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related to *MEN1* gene mutations, we analyzed the *MEN1* gene in this patient. A possible alteration of calcium metabolism associated with primary aldosteronism was also examined in this study.

Case Report

A 44-year-old Japanese woman was admitted to our hospital on May 9, 1995, to undergo an operation for left breast cancer. She had no serious illness before, but complained of occasional left flank pain for the past three months. There was no known family history of MEN1. On admission, her blood pressure was 192/118 mmHg with a pulse rate of 86 beats/min. Palpation of the left breast revealed a firm nodule of the size of 1.5-cm in diameter. The remainder of physical examination was not remarkable.

Laboratory examinations showed normal hemogram and normal liver function. Urine evaluation did not show any abnormalities. Serum albumin was 3.7 g/dl, blood urea nitrogen was 8.5 mg/dl, and creatinine was 0.7 mg/dl. A 24-hour creatinine clearance was estimated to be 77.4 ml/min. Serum electrolyte values were as follows: sodium, 146 mEq/l; potassium, 3.1 mEq/l; and chloride, 108 mEq/l. Serum calcium ranged from 10.2 to 10.4 mg/dl, while phosphate from 2.1 to 2.4 mg/dl. Urinary calcium excretion ranged from 138 to 203 mg/day. Serum alkaline phosphatase was not elevated. No abnormal findings were observed in chest and abdominal X-ray films. An electrocardiogram revealed the presence of prominent U waves.

The patient had a definitely elevated plasma aldosterone concentration (29 ng/dl, normal range: 3–16 ng/dl). Plasma renin activity, on the other hand, was very low, and showed a limited increase (from less than 0.1 to 0.2 ng/ml/h) after stimulation with an intravenous injection of 20 mg furosemide followed by standing for 2 hours. In addition, her plasma intact PTH level was 93 pg/ml, which clearly exceeded the normal range (14–66 pg/ml). Basal plasma levels of other hormones including growth hormone, thyroid stimulating hormone, prolactin, ACTH, thyroxine, cortisol, gastrin, glucagon, and insulin were all within normal limits. She had normal fasting blood glucose.

Ultrasonography of the neck revealed enlargement of the left superior parathyroid gland, which was confirmed by a CT scan. ¹³¹I-adosterol scintigraphy during dexamethasone suppression showed an asymmetric uptake in the left adrenal region. A CT scan of the abdomen demonstrated a left adrenocortical tumor of 2×3 cm in size. Based on these findings, a diagnosis of primary hyperparathyroidism associated with an aldosterone-producing adrenocortical adenoma was made.

Blood pressure was controlled with spironolactone and nifedipine, and left mastectomy was successfully done for breast cancer on June 8, 1995. The pathological diagnosis was scirrhous breast carcinoma (Fig. 1A). After the initiation of spironolactone therapy, her serum calcium gradually and progressively rose to higher levels (10.4 mg/dl before

spironolactone and 11.6 mg/dl after 8 weeks of spironolactone therapy), while plasma intact PTH levels were not significantly changed (93 pg/ml before spironolactone and 86 pg/ml after 8 weeks of spironolactone therapy). Left superior parathyroidectomy and left adrenalectomy were then performed simultaneously on December 8, 1999. An enlarged parathyroid gland of 2.1 g in weight, and an adrenocortical tumor were removed. The pathological diagnoses of the resected parathyroid gland and adrenal gland were parathyroid chief cell adenoma and adrenocortical adenoma with hyperplasia of zona glomerulosa, respectively (Fig. 1B and 1C). After the operation, serum calcium levels as well as plasma intact PTH declined to within the normal range. She was in good condition when she visited our outpatient clinic on April 10, 2003. She had normal serum potassium and calcium levels, and had no evidence of MEN-related neoplasia.

Materials and Methods

MEN1 gene mutation analysis

Blood was obtained from the patient after receiving written informed consent. Genomic DNA extraction from blood and *MEN1* gene mutation analyses were performed as previously described (8).

Parathyroid adenoma and adrenocortical adenoma were obtained from the patient during surgery. Fresh tissues were snap frozen in liquid nitrogen and stored at –70°C until later molecular analysis. Breast cancer tissue was embedded in paraffin. DNA was extracted either with a QIAamp tissue kit (Qiagen, Hilden, Germany) (parathyroid adenoma and adrenocortical adenoma) or with a TaKaRa DEXPAT™ (TaKaRa, Tokyo, Japan) (breast cancer) and amplified by PCR. Nucleotide sequencing was performed using a direct sequencing kit (Dye Terminator Cycle Sequencing Ready Reaction, Perkin-Elmer, Foster City, CA, USA) and an automated DNA sequencer (ABI PRISM 310, Perkin-Elmer).

LOH analysis

Microsatellite length polymorphism around the *MEN1* locus was analyzed using polymorphic DNA markers on chromosome 11q13, *PYGM* and *D11S4940*, as described previously (8). *PYGM* and *D11S4940* are located in centromeric and telomeric region, respectively, to the *MEN1* gene (Fig. 2). Genomic and tissue DNA were amplified by PCR with a pair of primers, one of which was labeled with fluorescein. The amount and the size of the PCR products were determined by electrophoresis followed by measurement of fluorescence intensity using an automated genetic analyzer (ABI PRISM 310, Perkin-Elmer).

Results

Germline *MEN1* gene mutation was detected at codon 541 in exon 10 of the *MEN1* gene that resulted in an alanine to

Figure 1. Photomicrographs of the tumor tissues obtained at surgery. A: breast cancer scirrhus, B: parathyroid tumor chief cell adenoma, C: adrenocortical tumor chief cell adenoma with hyperplasia of zona glomerulosa. A: HE stain ($\times 10$), B: HE stain ($\times 20$), C: HE stain ($\times 20$).

Figure 2. Schematic diagram of chromosome 11q13 illustrating the relative positions of two polymorphic markers (closed boxes). Positions of the *MEN1* gene (open box), and codon 541 in exon 10 are also shown. The distance between *PYGM* and *D11S4940* is less than 300 kb.

tyrosine substitution (A541T) in this patient (Fig. 3A). Microsatellite polymorphism analysis revealed reduced fluorescence intensity at each polymorphic site (*PYGM* and *D11S4940*) for one of the alleles in DNA from the parathyroid adenoma (Fig. 4B). The reduced fluorescence intensity site was found only at the *D11S4940* polymorphic site for one of the alleles in the breast cancer tissue DNA (Fig. 4D). Direct sequencing analyses of DNA from the parathyroid adenoma and breast cancer tissue again showed a mutation at codon 541 in exon 10 of the *MEN1* gene with a LOH in each contralateral allele (Fig. 3B and 3D). The latter finding is in

agreement with the result of the LOH study. On the other hand, LOH of the *MEN1* locus was not detected in DNA from the adrenocortical adenoma tissue (Fig. 4C).

Discussion

To determine whether the development of each tumor may be associated with an alteration of the *MEN1* gene, we analyzed the *MEN1* gene in a patient with a parathyroid adenoma, an aldosterone producing adrenocortical tumor and breast cancer in the present study. Germline *MEN1* gene mu-

Figure 3. Direct sequence analyses showing a benign polymorphism at codon 541 in exon 10 of the *MEN1* gene and a loss of the wild-type allele in the parathyroid adenoma and breast cancer tissue. A: Blood cell DNA; B: DNA from parathyroid tumor tissue; C: DNA from adreno-cortical tumor tissue; D: DNA from breast cancer tissue. Nucleotide position 541 is indicated by an arrow.

tation was detected at codon 541 in exon 10 of the *MEN1* gene in this patient. The incidence of the mutation is reported to be 24% in normal Japanese volunteers (9), thus it has been clearly demonstrated that this mutation is a benign polymorphism. Direct sequencing analyses of DNA from parathyroid adenoma, adrenocortical adenoma and breast cancer tissue again failed to show a somatic mutation in the protein-coding region of the *MEN1* gene. Microsatellite polymorphism analysis near the *MEN1* gene, however, demonstrated LOH in the parathyroid adenoma at both centromeric and telomeric regions to the *MEN1* gene. LOH was found also in breast cancer tissue when the polymorphic DNA marker telomeric to the *MEN1* gene was used. These results of the LOH study were in agreement with the direct sequencing analyses showing a LOH. The LOH of *MEN1* gene was seen in each contralateral allele of parathyroid adenoma and breast cancer. We conclude from these observations that an alteration of the *MEN1* gene and/or another tumor suppressor gene located in the *MEN1* locus on chromosome 11q13 may be involved in the development of parathyroid adenoma and breast cancer in the present patient suggesting that the clinical spectrum of *MEN1* might include breast cancer. To date, LOH of the *MEN1* locus in breast cancer as demonstrated in the present study has not been reported.

In the majority of *MEN1* patients, a wide variety of germline mutations of the *MEN1* gene has been demonstrated thus supporting the Knudson's two-hit hypothesis. Some reasons are suggested why *MEN1* gene mutations were not found in the parathyroid adenoma or the breast cancer with LOH of the *MEN1* gene. First, tumors may contain a mutation in the non-coding region of the *MEN1* gene that was not screened. Second, there may be an alternative mechanism, such as hypermethylation of a CpG island, responsible for LOH (10). Third, another tumor suppressor gene located near the *MEN1* gene on 11q13 may be involved in the development of tumors. In fact, LOH of the *MEN1* gene (6) or other tumor suppressor genes (10) without somatic gene mutations, as in the present study, has been reported in other tumors such as sporadic parathyroid adenomas and clear-cell renal carcinomas. Which of the above explanations may be correct for the present patient is unknown at present.

Although *MEN1* is characterized by parathyroid, pancreatic neuroendocrine, and pituitary hyperplasia or neoplasia, it is well known that tumors may occur in a variety of organs apparently unrelated to MEN in this pathologic state. One of the rare associations with *MEN1* is primary aldosteronism. In earlier reports, Beckers et al (11) and Iida et al (12) studied such patients and found LOH of the 11q13 locus in aldosterone-producing adrenocortical adenomas. They concluded from these results that primary aldosteronism in their patients may be concomitant with *MEN1*. In the present study, LOH of the *MEN1* locus was not demonstrated in the adrenocortical adenoma. These results suggest that the association of primary aldosteronism in our patient is incidental and is not related to *MEN1*.

Figure 4. Microsatellite polymorphism analysis using polymorphic DNA markers, *PYGM* and *D11S4940*. **A:** Blood cell DNA; **B:** DNA from parathyroid tumor tissue; **C:** DNA from adreno-cortical tumor tissue; **D:** DNA from breast cancer tissue. Arrows denote peaks with reduced fluorescence intensity in tumor compared with blood DNA.

Finally, the serum calcium concentration rose to higher levels after the initiation of spironolactone therapy in the present patient, although plasma intact PTH levels were not significantly changed. This may have resulted, in part, from the blockade by spironolactone of aldosterone actions. Excessive aldosterone induces an expansion of extracellular fluid, which, in turn, may inhibit the tubular reabsorption of calcium in the kidney, thus increasing urinary calcium excretion. Hyperaldosteronism causes hypokalemic alkalosis, which may decrease serum ionized calcium (13). Both

changes may lead to lowered serum calcium levels. Of relevance are studies by Resnick and Laragh (14), and by Rossi et al (15). They reported that serum calcium levels tend to be low, and plasma PTH levels tend to be high in patients with primary aldosteronism. The abnormalities were corrected after administration of spironolactone or surgical treatment (15), suggesting that primary aldosteronism is associated with mild secondary hyperparathyroidism. Coupled with these observations, serum calcium levels should be interpreted with caution in patients with primary aldosteronism, because hypercalcemia may be masked in the presence of aldosterone excess.

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