Humoral hypercalcemia associated with gastric carcinoma secreting parathyroid hormone: a case report and review of the literature

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Abstract. Hypercalcemia with concomitant elevation of serum parathyroid hormone (PTH) and PTH-related protein (PTHRP) levels was found in a patient with advanced gastric carcinoma and multiple liver metastases. The most common features are hypercalcemia associated with hypersecretion of PTHrP and physiological suppression of PTH secretion in the syndrome of humoral hypercalcemia of malignancy (HHM). Although we initially made a diagnosis of primary hyperparathyroidism concomitant with HHM due to gastric cancer, diagnostic imaging studies, such as echography, CT, sestamibi scintigraphy, and autopsy findings, did not reveal evidence of any parathyroid tumors or ectopic parathyroid glands in the mediastinum. Both primary and metastatic tumor cells showed positive staining with PTH-specific antibody as well as PTHrP-specific antibody on immunohistochemical examination. PTH concentration in the cytosolic fraction of the metastatic tumor was elevated compared to that from a control patient with no calcium metabolic disorders in vitro.

These findings indicated that PTH secreted ectopically by gastric cancer cells, not by parathyroid glands, caused hypercalcemia in this patient. To our knowledge, this is the first case report of PTH-secreting gastric carcinoma cells. We report the case and a review of the previous reported PTH-secreting non-parathyroid tumors along with the mechanisms of secretion.

Key words: Humoral hypercalcemia of malignancy (HHM), Parathyroid hormone, Gastric carcinoma, Immunohistochemistry, Cytosolic fraction
Materials and Methods

Hormone measurements

Serum full-length (1-84) PTH (whole PTH) and PTHrP concentrations were measured by two-site immunoradiometric assay (IRMA) (BML Co., Saitama, Japan). Two antibodies to N-terminal (1-6) PTH and (39-84) PTH were used for measurement of whole PTH concentration.

Immunohistochemistry

Immunohistochemistry of PTH and PTHrP was performed using standard techniques on formalin-fixed, paraffin-embedded sections of specimens of the primary tumor and liver metastases obtained at autopsy. Rabbit anti-human PTHrP polyclonal antibody (Oncogene Science, MA) and two different anti-PTH antibodies — rabbit anti-human PTH polyclonal antibody (Sigma-Aldrich, Tokyo, Japan) and mouse anti-human PTH monoclonal antibody (Novocastra, Wetzlar, Germany) — were used in these preparations, respectively.

Preparation of crude cytosolic fraction from the tissues

Metastatic liver tumor tissue was obtained from the patient at autopsy. A normal liver tissue biopsy specimen was obtained with permission from a control patient who had normal serum calcium concentration and normal serum PTH level. Each tissue was homogenized in a glass-Teflon homogenizer after addition of 1× PBS to prepare 10% w/v homogenate. Each homogenate was centrifuged at 1000 × g for 20 min at 4°C. The supernatants were used for measurement of whole PTH and PTHrP concentrations by two-site IRMA (BML Co.). This study was approved by the Institutional Review Board of Iida Municipal Hospital.

Case Report

A 70-year-old man was admitted to our hospital in May 2011 because of general fatigue, upper abdominal tenderness, and thirst. He had no history of serious illness and no family history of endocrine diseases. On admission, he complained of upper abdominal tenderness, and his liver was palpable in the epigastric region. Laboratory data after admission are summarized in Table 1. Serum calcium level was 14.8 mg/dL and serum inorganic phosphorus level was 1.8 mg/dL. Whole PTH level was 190 pg/mL (normal range: 9 – 39 pg/mL) or 20 pmol/L (normal range: 0.95 – 4.1 pmol/L). PTHrP level was 3.8 pmol/L (normal range: <1.1 pmol/L). CEA was 1015 ng/mL and CA19-9 was 187.7 U/mL. Computed tomography (CT) scan indicated that he had a mass measuring approximately 3 cm in the stomach (Fig. 1, arrows) and multiple metastatic tumors in the stomach.

Table 1 Laboratory Findings

<table>
<thead>
<tr>
<th></th>
<th>on admission</th>
<th>17 days after</th>
<th>43 days after</th>
<th>normal range</th>
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<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>3.1</td>
<td>2.8</td>
<td>2.1</td>
<td>3.8-5.3</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.1</td>
<td>1.8</td>
<td>5.5</td>
<td>0.3-1.2</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>129</td>
<td>260</td>
<td>655</td>
<td>12-37</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>51</td>
<td>104</td>
<td>301</td>
<td>7-45</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1394</td>
<td>1959</td>
<td>2357</td>
<td>114-220</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>1395</td>
<td>2499</td>
<td>3434</td>
<td>124-367</td>
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<tr>
<td>γ-GTP (U/L)</td>
<td>734</td>
<td>942</td>
<td>8-50</td>
<td></td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>14.8</td>
<td>11.2</td>
<td>13.0</td>
<td>8.6-10.1</td>
</tr>
<tr>
<td>IP (mg/dL)</td>
<td>1.8</td>
<td>1.9</td>
<td>2.2-4.1</td>
<td></td>
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<tr>
<td>Whole PTH (pg/mL)</td>
<td>190</td>
<td>318</td>
<td>9-39</td>
<td></td>
</tr>
<tr>
<td>PTHrP (pmol/L)</td>
<td>3.8</td>
<td>&lt;1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>19.7</td>
<td>17.7</td>
<td>30.5</td>
<td>9-22</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.97</td>
<td>0.83</td>
<td>0.85</td>
<td>0.6-1.1</td>
</tr>
<tr>
<td>CEA (ng/mL)</td>
<td>1015</td>
<td>&lt;5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA19-9 (U/mL)</td>
<td>187.7</td>
<td>&lt;37</td>
<td></td>
<td></td>
</tr>
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</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; Ca, calcium; IP, inorganic phosphate; PTHrP, parathyroid hormone related protein; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9
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CT scan taken on admission. Gastric tumor (arrows) and multiple liver tumors were detected.

Endoscopic examination of the upper gastrointestinal tract indicated advanced type 3 gastric cancer at the posterior wall of the antrum. Histological diagnosis of biopsy specimens was gastric adenocarcinoma. Our initial diagnosis was gastric carcinoma with hypercalcemia due to HHM. Primary hyperparathyroidism was another diagnosis because of hypercalcemia with hypersecretion of PTH. To detect the parathyroid tumor, we performed imaging studies for primary parathyroidism, including $^{99m}$Tc sestamibi scintigraphy, echography, and CT scan of the neck. There was no evidence of parathyroid gland enlargement. As his gastric cancer was interpreted as inoperable, he received chemotherapy with fluoropyrimidine, and zoledronic acid hydrate repeatedly to improve hypercalcemia during the period of hospitalization. His serum calcium level was reduced only transiently by zoledronic acid hydrate. Prednisolone and elcatonin were started as additional therapies for hypercalcemia.

Eventually, the patient died due to multiorgan failure on the 44th day after admission and autopsy was performed 1 h after death. Grossly, type 3 gastric cancer was seen in the posterior wall of the antrum. Histologically, gastric cancer showed a distinct tubular structure with a partially poorly differentiated component. Liver tumors were histologically similar to those in the stomach, suggesting that they were metastases of gastric adenocarcinoma. Four normal-sized parathyroid glands were identified. No ectopic parathyroid glands were found in the mediastinum. The results of histopathological examination for these glands were all normal. Immunohistochemically, tumor cells of the stomach and liver showed positive staining for PTHrP (Fig. 2, C, D). Immunohistochemically, some tumor cells showed positivity for PTH (Fig. 2, A, B) as well as PTHrP (Fig. 2, C, D), but the number of PTH-positive cells was far less than that of PTHrP-positive cells. Some cells in the normal gastric mucosa of this patient showed positivity for PTHrP (Fig. 2, E).

We stained the tissues with the two different antibodies that recognize different epitopes of the PTH molecule. The same tissues were stained with both rabbit anti-human PTH polyclonal antibody and mouse anti-human PTH monoclonal antibody (data not shown). Next, we prepared the crude cytosolic fraction from metastatic liver tumor and measured the concentration of whole PTH to confirm that the tumor had produced PTH. We also measured the concentration of PTHrP. Whole PTH and PTHrP concentrations in the crude cytosolic fraction of metastatic liver tumor were 298 pg/mL and 17.2 pmol/L, respectively. Whole PTH and PTHrP levels in the control were 23 pg/mL and 6.4 pmol/L, respectively (Table 2).

**Discussion**

The most common features of HHM are hypercalcemia associated with overexpression of PTHrP accompanied with suppressed PTH secretion. The tumor cells showed strong positive staining for PTHrP, in contrast to weak positive in the normal gastric mucosa in this case. The PTHrP level in the crude cytosolic fraction of metastatic liver tumor was elevated by approximately 2.7-fold compared with liver tissue from a control patient whose serum PTHrP was below the limit of detection (Table 2). These data suggested that abnor-
Table 2  Whole PTH and PTHrP concentration in the crude cytosolic fraction

<table>
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<tr>
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<th>Whole PTH (pg/mL)</th>
<th>PTHrP (pmol/L)</th>
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<tbody>
<tr>
<td>this case</td>
<td>298</td>
<td>17.2</td>
</tr>
<tr>
<td>control</td>
<td>23</td>
<td>6.4</td>
</tr>
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</table>

control: liver tissue obtained from a control patient whose serum calcium was 9.0mg/dL, serum whole PTH level was 30pg/mL and serum PTHrP was undetectable.

Fig. 2  Immunohistochemical examination of the gastric and liver tumors. Representative views of PTH staining of gastric carcinoma with rabbit anti-human PTH polyclonal antibody (A) and liver metastatic tumor with mouse anti-human PTH monoclonal antibody (B). (×400). Note that scattered cells showed positive staining for PTH (arrows) in the original tumor and metastatic liver tumors. Gastric tumor and liver tumors were both positive for PTHrP (C, D). Scattered cells in the normal gastric mucosa of this patient showed positive staining for PTHrP (E, arrows). (×400).
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mal PTHrP secretion from tumor cells may have been one of the causes of HHM in this case.

HHM due to hypersecretion of PTH is rare. There have been reports of ectopic authentic PTH production in nonparathyroid malignancy, the lung [5, 6, 12, 18], ovary [7], uterus [16], liver [17], thymus [8], thyroid gland [9, 19], tonsil [13], kidney [14], pancreas [11, 15], or neuroectodermal tissue [10]. In general, the measurement of serum PTH is routinely performed to exclude the diagnosis of primary hyperparathyroidism in the presence of hypercalcemia. The patient in this case initially showed classical biochemical features of primary hyperparathyroidism concomitant with HHM. However, clinical and pathological findings, including autopsy findings, indicated no evidence of parathyroid tumor. Therefore, we speculated that the malignant tumor may have secreted not only PTHrP but also PTH in this case.

Positive staining for PTH was observed in several cells of the primary lesion (Fig. 2, A), as well as in the metastatic lesions (Fig. 2, B). To confirm inappropriate secretion of PTH from the tumor, we measured PTH level in the crude extract of tumor tissues. Whole PTH level in the crude cytosolic fraction of metastatic liver tumor was highly elevated by approximately 13-fold compared with the liver tissue obtained from a control patient who had normal serum whole PTH and calcium levels (Table 2), indicating that PTH as well as PTHrP were secreted from the tumor cells. Accordingly, we concluded that the disproportionate elevation of PTH may have been due to inappropriate secretion of PTHrP and PTH from the tumor cells and not from the parathyroid glands in this case. The measurement of PTH concentration in the crude cytosolic fraction of the tumor tissue is useful for diagnosis of ectopic PTH production when the diagnosis of primary hyperparathyroidism is less likely. Thus, we concluded that PTH was abnormally produced by the cells of gastric carcinoma and resulted in the development of HHM in this case.

PTHrP is a protein of 139 – 173 amino acids, which has the significant sequence homology of N-terminal end as PTH. HHM associated with PTHrP may occur in patients with malignancies originating from a range of tissues [3]. PTHrP (1-141) reduces bicarbonate excretion while PTH or PTHrP (1-34) does not [20], and HHM patients have a metabolic alkalosis rather than hyperchloremic acidosis [2, 21]. Moreover, serum 1,25-dihydroxy vitamin D3 concentrations are increased in primary hyperparathyroidism and suppressed in HHM [2, 21]. Therefore, low plasma level of 1,25-dihydroxy vitamin D3 or presence of alkalosis might be helpful for speculating on contribution of PTHrP rather than PTH, although these data were not available in this case. As the molar concentration of serum whole PTH was approximately 5 times higher than that of PTHrP on admission, ectopic PTH secretion may potentially contribute to the hypercalcemia in this case.

Several reports have documented that various tumor cells expressed the PTH mRNA and speculated on the molecular mechanisms underlying the ectopic production of PTH [5, 7, 10, 11, 19]. In addition, it is intriguing to note that immunohistochemistry for PTH was not obvious in a case of sporadic medullary thyroid cancer, while RT-PCR confirmed ectopic PTH gene expression [19]. The arrangements of the PTH gene promoter with the cyclin D1 gene in parathyroid adenomas suggest that DNA sequences necessary for tissue-specific expression of the PTH gene reside in the 5' regulatory region of the gene [22]. A report of ovarian cancer documented the molecular etiology of PTH gene expression, i.e., amplification and rearrangement of the 5' regulatory region of the PTH gene [7]. However, other investigators could not detect definitive molecular modifications, and they speculated on various mechanisms for ectopic PTH expression [5, 10, 11, 19]. Thus, ectopic PTH production may involve heterogeneous molecular mechanisms.

In conclusion, we reported the first case of HHM due to PTH-secreting gastric carcinoma and presented a review of previously reported PTH-secreting carcinomas and the molecular mechanisms of inappropriate expression of the gene. It is important and necessary to measure not only serum PTHrP levels but also serum PTH levels in HHM patients. In addition, measuring PTH concentration in the crude cytosolic fraction of tumor tissue is useful for estimation of ectopic PTH production from tumor cells.

Acknowledgement

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Conflict of Interest

All authors have nothing to declare.
References