Circulating Parathyroid Hormone-related Protein (109-141) in Malignancy-associated Hypercalcemia

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For differential diagnosis between hypercalcemia-induced bone metastasis and humoral hypercalcemia of malignancy (HHM), serum parathyroid hormone-related protein (PTHrP) concentrations were measured in normal subjects and patients with malignancy-associated hypercalcemia according to the presence or absence of bone metastasis, using a new sensitive PTHrP(109-141) radioimmunoassay system. The serum PTHrP(109-141) levels in all of 14 patients without bone metastasis were significantly higher than those in normal subjects. However, in four patients with hypercalcemia associated with bone metastasis the levels were nearly the same as those in normal subjects. The time course in two hypercalcemic patients with esophageal carcinoma revealed that serum PTHrP(109-141) levels were elevated before hypercalcemia developed and that changes in PTHrP(109-141) and corrected serum calcium levels were significantly correlated. These findings suggest that determination of serum PTHrP(109-141) may be clinically important not only for differential diagnosis of HHM but also as a useful predictive marker of hypercalcemia.

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Key words: Hypercalcemia—Parathyroid hormone-related protein—Paraneoplastic syndrome—Tumor marker—Esophageal carcinoma

Introduction

Hypercalcemia is a common paraneoplastic syndrome that may develop in cancer patients. This electrolyte imbalance can be divided into two categories based on its etiology; one form is due to severe bone metastasis and is also termed local osteolytic hypercalcemia, and the other, due to the elaboration of hypercalcemic factors by solid tumors, is termed "humoral hypercalcemia of malignancy (HHM)". It is roughly estimated that more than 80% of malignancy-associated hypercalcemias are HHM. Although the original concept of HHM was limited to solid tumors, hypercalcemia frequently associated with adult T-cell leukemia is also considered to belong to the HHM category. Although factors with bone-resorbing activity, including parathyroid hormone (PTH), transforming growth factor-α and -β, interleukin-α and -β, epidermal growth factor and prostaglandins, have been claimed to be candidates for the humoral factors responsible for HHM, previous studies including ours have revealed that PTH-related protein (PTHrP) is the major factor causing HHM.

PTHrP has been isolated from several malignant tumors causing HHM, and was cloned in 1987.1-3 The amino-terminal portion of PTHrP has significant sequence homology with the corresponding region of PTH and interacts with the PTH receptor, demonstrating biological activity similar to that of PTH. Amino- and carboxy-terminal immunoassays for measuring serum PTHrP levels have been reported, and the results of the assays suggest that circulating PTHrP may contribute to the pathogenesis of hypercalcemia in some patients, even though the circulating forms of PTHrP are still incompletely characterized.4,5 However, the previous assay systems are not sensitive enough, and serum PTHrP in normal subjects is almost undetectable. Recently a specific carboxy-terminal assay for serum

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PTHrP was developed and shown to be capable of detecting PTHrP(109–141) in normal subjects. To evaluate the usefulness of the PTHrP(109–141) radioimmunoassay (RIA) system, we measured serum PTHrP levels in normal subjects and patients who had hypercalcemia associated with malignancy.

Patients and Methods

Patients

Serum samples were obtained with informed consent from 18 patients with malignancy-associated hypercalcemia (corrected serum calcium > 10.5 mg/dl), none of whom had renal failure; corrected serum calcium levels were calculated according to the equation described by Mundy and Martin. Of the 18 patients, 14 developed hypercalcemia without bone metastasis, as confirmed by negative findings of bone scintigraphy and/or bone roentgenography. These patients included seven with esophageal carcinoma, three with breast cancer, two with carcinoma of the tongue, and one each with islet cell carcinoma of the pancreas and carcinoma of the neck. The other four patients were diagnosed as having hypercalcemia associated with diffuse bone metastasis by bone scintigraphy; three of them had breast cancer and one had malignant mesenchymoma (Table I).

The time courses of serum PTHrP and corrected serum calcium levels from the date of admission to death were examined in two esophageal carcinoma patients who had advanced disease which was deemed inoperable. Patient A was a 72-year-old man who was admitted to our hospital in August 1990, and patient B was a 54-year-old man who was admitted in April 1991. These two patients were treated by combined chemotherapy. The efficacy of this therapy was evaluated by an imaging diagnostic technique and the overall response was analyzed according to the WHO criteria. Patient A achieved a partial response (PR) in 60 days and was judged as having progressive disease (PD) 137 days after being admitted. Combined chemotherapy was not effective in patient B, who had been judged as having PD at 50 days.

Ninety-eight normal subjects examined were laboratory and hospital staff members with normal serum calcium levels. None of them had renal failure. Collected serum samples without protease inhibitors were stored at -20°C for measurement of PTHrP(109–141) concentration.

PTHrP RIA

The PTHrP RIA was performed using antiserum against human PTHrP(109–141) provided by Daiichi Radioisotope Laboratories, Ltd., Tokyo, as reported previously. PTHrP(109–141) was used as

### Table I. Corrected Serum Calcium and PTHrP(109–141) Levels in Patients with Hypercalcemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Site of malignancy</th>
<th>Bone metastasis</th>
<th>Serum calcium (mg/ml)</th>
<th>Serum PTHrP (pmol/l)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>14.4</td>
<td>213.2</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>14.1</td>
<td>233.9</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>13.9</td>
<td>575.8</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>13.7</td>
<td>425.0</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>13.5</td>
<td>167.6</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>13.5</td>
<td>126.0</td>
<td>0.8</td>
</tr>
<tr>
<td>7</td>
<td>51</td>
<td>M</td>
<td>Esophagus</td>
<td>(–)</td>
<td>13.2</td>
<td>765.6</td>
<td>1.2</td>
</tr>
<tr>
<td>8</td>
<td>39</td>
<td>F</td>
<td>Breast</td>
<td>(–)</td>
<td>16.7</td>
<td>161.2</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>F</td>
<td>Breast</td>
<td>(–)</td>
<td>12.7</td>
<td>148.3</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>F</td>
<td>Breast</td>
<td>(–)</td>
<td>11.9</td>
<td>1454.0</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>77</td>
<td>F</td>
<td>Tongue</td>
<td>(–)</td>
<td>14.2</td>
<td>438.4</td>
<td>0.9</td>
</tr>
<tr>
<td>12</td>
<td>66</td>
<td>F</td>
<td>Tongue</td>
<td>(–)</td>
<td>13.2</td>
<td>252.9</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>55</td>
<td>F</td>
<td>Neck</td>
<td>(–)</td>
<td>13.6</td>
<td>106.3</td>
<td>0.5</td>
</tr>
<tr>
<td>14</td>
<td>38</td>
<td>M</td>
<td>Islet cell</td>
<td>(–)</td>
<td>13.4</td>
<td>293.0</td>
<td>0.7</td>
</tr>
<tr>
<td>15</td>
<td>57</td>
<td>F</td>
<td>Breast</td>
<td>(+)</td>
<td>14.0</td>
<td>68.6</td>
<td>1.2</td>
</tr>
<tr>
<td>16</td>
<td>44</td>
<td>F</td>
<td>Breast</td>
<td>(+)</td>
<td>13.0</td>
<td>57.5</td>
<td>0.9</td>
</tr>
<tr>
<td>17</td>
<td>43</td>
<td>F</td>
<td>Breast</td>
<td>(+)</td>
<td>11.6</td>
<td>37.5</td>
<td>0.6</td>
</tr>
<tr>
<td>18</td>
<td>17</td>
<td>M</td>
<td>Malignant mesenchymoma</td>
<td>(+)</td>
<td>17.9</td>
<td>72.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Serum PTHrP(109-141) levels (pmol/l)

<table>
<thead>
<tr>
<th>Normal subjects</th>
<th>HHM</th>
<th>Bone meta.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Serum immunoreactive PTHrP(109–141) levels in normal subjects and patients with hypercalcemia. The dashed line indicates the detection limit of the assay. Bone meta, hypercalcemia associated with bone metastases.

the assay standard and $^{125}$Tyr-PTHrP(108–141) as the tracer. A 0.2-ml serum sample was used directly for the assay. It showed virtually no cross-reactivity with PTH.

Results

The serum PTHrP(109–141) levels in patients with hypercalcemia and normal subjects are shown in Fig. 1. The levels in normal subjects ranged from 15.4 to 69.3 pmol/l, with a mean of 29.6 ± 8.3 pmol/l (mean ± SD). The normal range was therefore set between 13.0 and 46.2 pmol/l (mean ± 2SD). When the upper limit of the PTHrP(109–141) level in the normal range was set at 46.2 pmol/l (mean ± 2SD), serum PTHrP(109–141) levels of all 14 hypercalcemic patients without bone metastasis were significantly elevated; the mean level was 382.9 ± 362.1 pmol/l, ranging from 106.3 to 1454 pmol/l. In contrast, the four patients with hypercalcemia associated with bone metastasis had lower values; the mean was 59.0 ± 15.7 pmol/l, ranging from 37.5 to 72.7 pmol/l. The PTHrP(109–141) concentrations in these patients are shown in Table I.

The time course of serum PTHrP(109–141) was examined in two patients with esophageal carcinoma. Corrected serum calcium levels in these two patients rose after the serum PTHrP levels started to increase (Fig. 2). It was noteworthy that in patient B the serum PTHrP(109–141) level exceeded the normal range one month before hypercalcemia developed. The two patients received combined chemotherapy. When PR was achieved in patient A on day 60, his serum PTHrP(109–141) level was decreased. The level was elevated on day 137, corresponding to the evaluation as PD. PTHrP levels were markedly elevated before death in both patients. The correlation between corrected serum calcium concentrations and serum PTHrP(109–141) concentrations is shown in Fig. 3. In both patients there was a significant correlation between these two variables ($r = 0.84, 0.97$ respectively).

Discussion

Recent studies including ours have demonstrated that the major factor responsible for HHM is PTHrP. It has also been shown that plasma PTHrP levels are elevated in HHM patients, although most of these studies measured the bioactive portion of PTHrP, which is not stable in the blood. Accordingly, it is necessary to add enzyme inhibitors at the time of blood collection, and plasma PTHrP levels in normal subjects are not detectable. In the present study, we used an assay system recognizing PTHrP(109–141), a carboxyl-terminal fragment of intact PTHrP. Although it was demonstrated that a carboxyl-terminal fragment of PTHrP was excreted in urine of patients with humoral hypercalcemia of malignancy,12 this molecule was also found to be stable in blood, thus making it possible to measure the serum PTHrP(109–141) level in all normal subjects.

Serum PTHrP levels in 14 hypercalcemic patients without bone metastasis were markedly higher than in normal subjects, and the values were always greater than 100 pmol/l. Serial determination of serum PTHrP(109–141) levels in two HHM patients with esophageal carcinoma revealed that at the time of initial evaluation, the levels were slightly elevated, 61.5 and 62.9 pmol/l respectively, although corrected serum calcium levels were within the normal range. In both patients, when serum PTHrP levels were markedly elevated before death in both patients. The correlation between corrected serum calcium concentrations and serum PTHrP(109–141) concentrations is shown in Fig. 3. In both patients there was a significant correlation between these two variables ($r = 0.84, 0.97$ respectively).
reached nearly 100 pmol/L, the corrected serum calcium levels were elevated. Thus, it is reasonable to speculate that the development of HHM could be divided into two stages. At the earlier stage, a slight increase in the serum PTHrP level preceded that of the serum calcium level. As for why the serum calcium level was not elevated, the following reason can be speculated. Slight elevation of the serum PTHrP level is not enough to stimulate bone resorption; alternatively, PTHrP stimulates bone resorption but an increase in the urinary secretion of calcium may prevent hypercalcemia. A further increase in the serum PTHrP level to more than 100 pmol/L then induced hypercalcemia. Hypercalcemia sometimes develops suddenly in patients with advanced cancer, and early clinical detection of this morbidity is needed. In these two esophageal carcinoma patients, serum PTHrP(109–141) levels were increased before hypercalcemia developed. From a clinical standpoint, it is possible to speculate that a slight increase in the serum PTHrP level indicates recurrence of the disease and is a predictor of hypercalcemia. These observations suggest that determination of serum PTHrP(109–141) levels could be useful for monitoring tumor progression or response to treatment, although further study in a number of such patients is required.

In contrast, PTHrP(109–141) levels in patients with hypercalcemia associated with bone metastasis were not significantly elevated. Although other humoral factors are considered to be involved in the pathogenesis of hypercalcemia, it is clear that the levels of serum PTHrP(109–141) in hypercalcemic patients without bone metastasis are significantly higher than those in patients with hypercalcemia with bone metastasis. These results indicate that measurement of serum PTHrP(109–141) is useful for differential diagnosis of HHM. In the future, it might be possible to use the serum PTHrP level for selecting patients eligible for new specific therapy modalities such as PTHrP inhibitors or antagonist. Although recent reports have shown that the serum PTHrP level, as measured by the carboxyl-terminal assay system, is higher in patients with impaired renal function, none of the hypercalcemic patients or normal subjects in this study had renal failure. Therefore, evaluation of data such as the creatinine level is required.

Although hypercalcemia associated with breast cancer has generally been considered to develop as a result of the osteolytic action of bone metastases, previous reports have suggested a humoral mechanism. In this study, the serum PTHrP(109–141) level in six breast cancer patients
with hypercalcemia was measured. The serum PTHrP(109–141) levels in three patients without bone metastases were significantly elevated, and of three patients with bone metastases, two had slightly elevated serum PTHrP(109–141) levels. In these patients, PTHrP produced by the tumor including the metastatic sites may have exerted no systemic effect, but it may have been important for local osteolysis at the sites of bone metastases. Recent studies indicate that PTHrP-producing cancer cells frequently detected at sites of bone metastases. Recent studies indicate that PTHrP-producing cancer cells frequently detected at sites of bone metastases and that PTHrP production by the tumor may facilitate the development of bone metastases in patients with breast cancer. 

In our patients, it was unclear whether PTHrP contributed to bone metastasis, but it is likely that humoral mechanisms are commonly involved, irrespective of the presence or absence of bone metastasis. At this stage, it appears that classification of hypercalcemic syndromes based on the presence or absence of bone metastasis is flawed.

In summary, determination of circulating C-terminal PTHrP(109–141) should be clinically important for differential diagnosis between HHM and hypercalcemia induced by bone metastases. In addition, it may be important in providing a useful marker for predicting sudden hypercalcemia and for monitoring the response to treatment in patients with malignancy.

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References

7) Grill V, Ho P, Body JJ, Johanson N, Lee SC, Kukreja SC, Moseley JM, Martin TJ: Parathyroid hormone-related protein: elevated levels in both hu-
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