© Acta Endocrinologica 1992, 127: 324–30

Serum parathyroid hormone-related protein concentrations in patients with hematologic malignancies or solid tumors

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Nakamura Y, Bando H, Shintani Y, Yokogoshi Y, Saito S. Serum parathyroid hormone-related protein concentrations in patients with hematologic malignancies or solid tumors. Acta Endocrinol 1992;127:324–30. ISSN 0001–5598

The clinical significance of parathyroid hormone-related protein in humoral hypercalcemia of malignancy was investigated by determining the serum parathyroid hormone-related protein concentrations in 167 normal subjects, 56 patients with hematologic malignancy and 144 patients with solid tumor. Serum parathyroid hormone-related protein was measured with a radioimmunoassay kit that recognizes the C-terminal portion of the molecule. The serum parathyroid hormone-related protein concentrations were 20.2–50.8 pmol/l (mean ± 2 SD) in normal subjects, and were elevated in 80% of the patients with malignancies with hypercalcemia, including squamous cell carcinoma and adult T cell leukemia. Moreover, two cases of B cell non-Hodgkin's lymphoma with hypercalcemia had high serum parathyroid hormone-related protein concentrations, which varied in parallel with the tumor size during the clinical course. Of 136 patients with solid tumors with normocalcemia, the serum parathyroid hormone-related protein concentration was slightly elevated in only 5.1%, all of whom were at an advanced stage. These data indicate that determination of the serum parathyroid hormone-related protein concentration is useful for differential diagnosis of humoral hypercalcemia of malignancy and prediction of its development.

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In 1987, four groups (1–4) purified parathyroid hormone-related protein (PTHrP) and Suva et al. (5) cloned and sequenced the gene of this protein. In addition, immunoreactive PTHrP was demonstrated in an extract of a humoral hypercalcemia of malignancy tumor (6, 7) by radioimmunoassay (RIA). In 1990, Burtis et al. (8) developed an immunoradiometric assay (IRMA) for PTHrP (1–74) and an RIA for PTHrP (109–138), and reported that both their assays were useful in the differential diagnosis of hypercalcemia. The value of determining urinary PTHrP (127–141) was also reported (9). However, the serum PTHrP levels in normal subjects are difficult to measure by these assays, and there have been few reports on the relationship between the clinical course and serum PTHrP level. Recently, Kasahara et al. (10) developed an RIA kit for PTHrP (109–141) with which we could measure the serum PTHrP concentrations in normal subjects and in patients with various diseases. Here we report data on the serum PTHrP concentrations in patients with hematologic malignancies or solid tumors who had normal serum creatinine levels, and the relationship between the clinical course and change in serum PTHrP.

Subjects and methods

Subjects

The subjects examined were 167 normal adults (92M, 75F) aged 20–60 years, 56 patients with hematologic malignancy and 144 patients with solid tumor.

Serum calcium was measured using the O-cresolphthalein complexing method after an overnight fast. The serum calcium concentration was corrected by the equation, "corrected calcium = measured calcium (mg/dl) + 4.0-albumin (g/dl)" and values in mg/dl were converted to mmol/l. Normal range of serum calcium was 2.25 ± 0.20 mmol/l (mean ± 2 SD). Hypercalcemia was defined as a serum calcium level of above 2.65 mmol/l (mean + 4 SD).

Serum creatinine was measured by Jaffé's test. No significant difference was observed between serum creatinine concentrations in normal subjects and tumor patients (mean ± 2 SD, 79.7 ± 26.6 vs 79.6 ± 17.7 μmol/l).

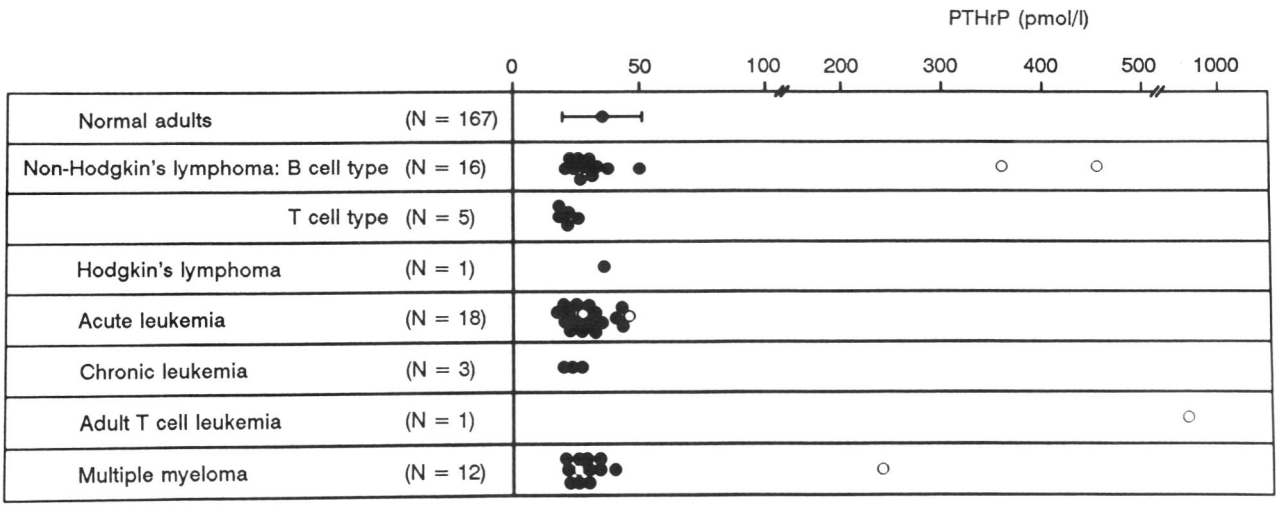

Fig. 1. Serum PTHrP concentrations in normal subjects and in patients with various hematologic malignancies. The bar indicates the mean ± 2 SD in normal subjects. Each point represents serum PTHrP concentration in patients with hypercalcemia (○) or normocalcemia (●).

Table 1. Clinical stage in the patients with solid tumor who had normocalcemia and elevation of serum PTHrP level.

Case	Age	Sex	Diagnosis	Stage	Ca (mmol/l)	PTHrP (pmol/l)
1	53	M	Oral cancer (SCC)	IV	2.23	64.0
2	52	F	Oral cancer (SCC)	IV	2.25	63.7
3	65	M	Oral cancer (SCC)	IV	2.18	58.2
4	67	M	Lung cancer (SCC)	IV	2.15	72.9
6	65	F	Lung cancer (SCC)	IV	2.35	88.7
6	72	M	Lung cancer (SCC)	IIIb	2.13	63.4
7	72	F	Prostatic cancer (adeno ca.)	IV	2.18	242.4

SCC: squamous cell carcinoma; adeno ca.: adenocarcinoma.

Determination of serum PTHrP

Serum PTHrP concentration was measured with a PTHrP kit (D-0102, Daiichi Radioisotope Institute, Tokyo, Japan) (10). The kit is composed of antibody raised against human PTHrP (109–141), ^{125}I-labelled [Tyr^{108}]-PTHrP (108–141) and synthetic human PTHrP (109–141) as a standard. As reported previously (10), 100 μl of ^{125}I-labelled PTHrP (108–141) and 100 μl of antibody were incubated with 200 μl of a standard solution or sample for 22 h. Then the second antibody (anti-sheep donkey IgG antibody) was added, and incubation was continued for 30 min. The mixture was then centrifuged, the supernatant removed by aspiration, and the radioactivity of the residue measured. By this assay the detectable range is 10–1000 pmol/l. The intra-assay and interassay coefficients of variation were found to be 1.91–5.94% and 2.75–3.82% at concentrations of 39 and 330 pmol/l, respectively, and the recoveries of PTHrP added to serum samples at concentrations of 15, 50, 150 and 500 pmol/l were 98.5 to 105.7%.

Statistical analysis

Student's *t*-test was used to compare serum PTHrP levels in two groups, and a p value of less than 0.05 was considered to indicate statistical significance.

Results

Serum PTHrP concentrations

Normal subjects. The serum PTHrP concentrations of 167 normal subjects were distributed lognormally in the range of 20.2–50.8 pmol/l (mean ± 2 SD) after logarithmic transformation. No significant difference was observed between the values of males and females.

Patients with hematologic malignancy. Of 56 patients with hematologic malignancy, 7 patients (2 with malignant lymphoma, 3 with leukemia, 2 with myeloma) had hypercalcemia. Of these patients, 2 with B cell non-Hodgkin's lymphoma, 1 with adult T cell leukemia and 1 with myeloma had high serum PTHrP concentrations (Fig. 1).

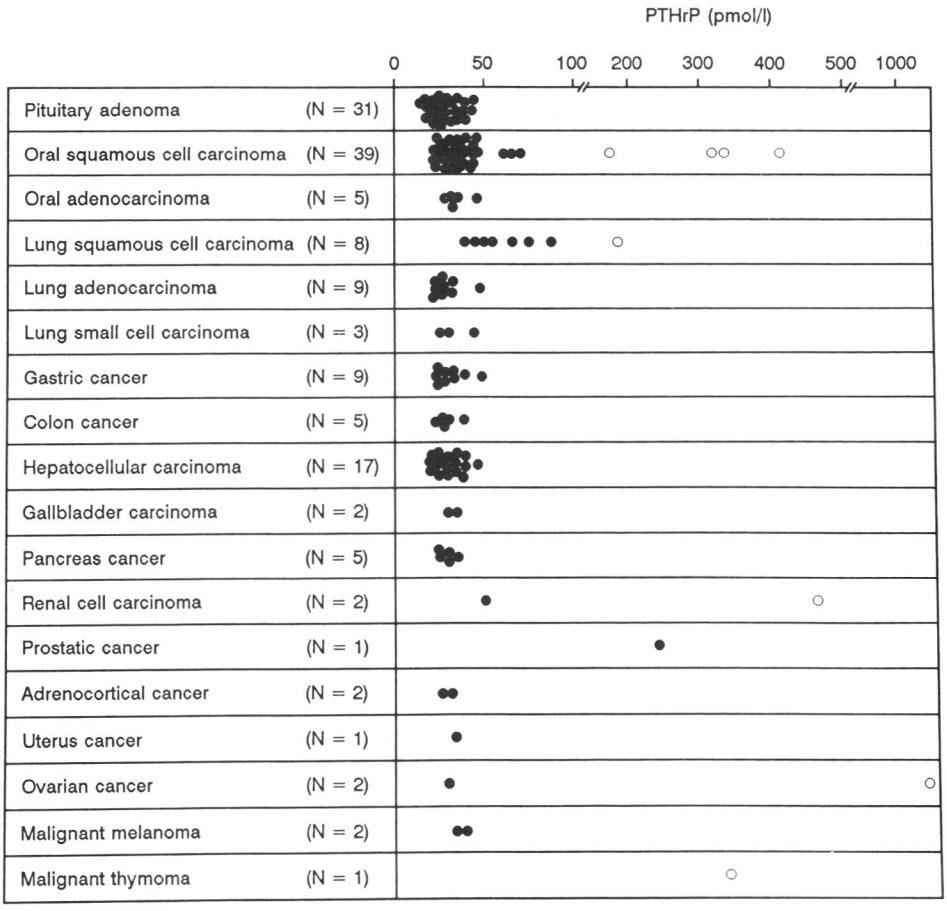

Fig. 2. Serum PTHrP concentrations in 144 patients with various solid tumors. Each point represents serum PTHrP concentration in patients with hypercalcemia (○) and normocalcemia (●).

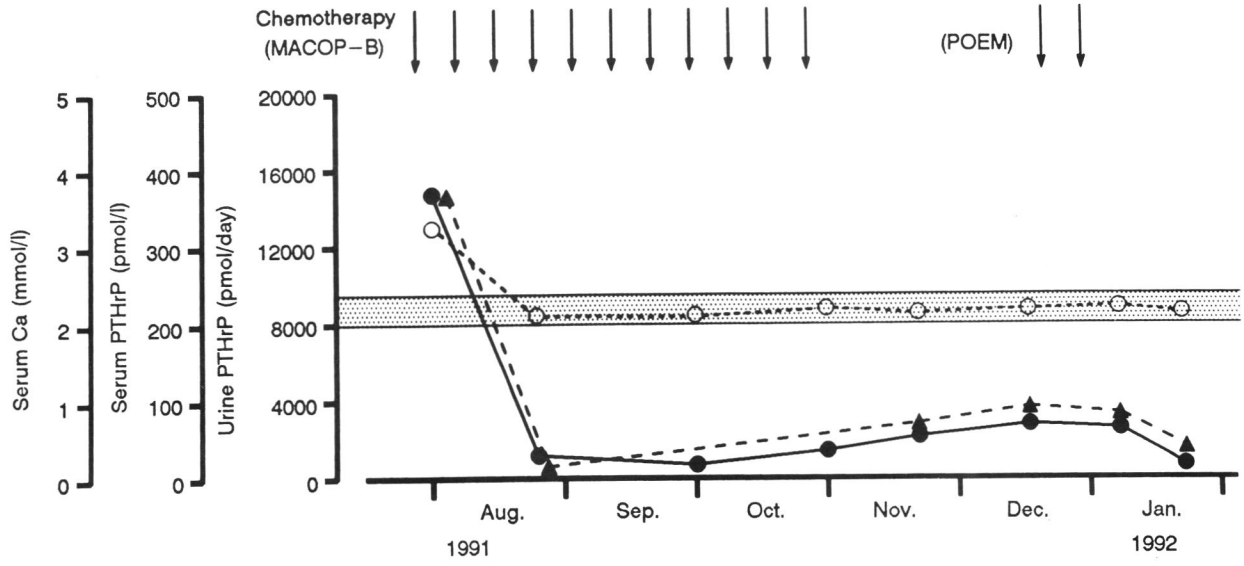

Fig. 3. Clinical course of Case 1. The levels of serum calcium (Ca) (○), serum PTHrP (●) and urinary PTHrP (▲) are shown. The shaded area represents the normal range of serum calcium level. MACOP-B: adriamycin, cyclophosphamide, vincristine, bleomycin and prednisolone. POEM: vincristine, etoposide, mitoxantrone and prednisolone.

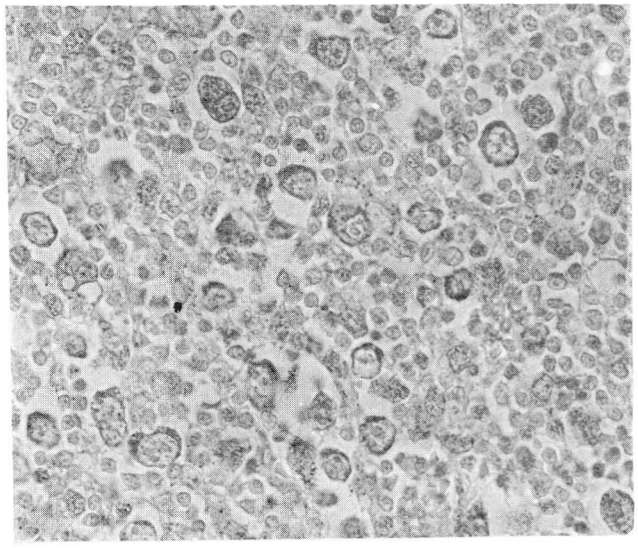

Fig. 4. Immunoperoxidase staining of a lymph node with anti PTHrP (34–53) antibody. Immunoactive PTHrP was found in the cytoplasm of the lymphoma cells (× 400).

Of 22 patients with malignant lymphoma, 2 patients with high serum PTHrP concentration were advanced cases at clinical stage IV.

Patients with solid tumor. Of 144 patients with solid tumor, 8 patients had hypercalcemia. All of them had high serum PTHrP concentrations.

Of 136 patients with solid tumors with normocalcemia, only 7 patients had slightly elevated serum PTHrP levels (Table 1).

In patients with lung cancer, the serum PTHrP concentration was 37.1–181.8 pmol/l in eight with squamous cell carcinoma, 20.2–48.1 pmol/l in nine with adenocarcinoma and 22.7–43.8 pmol/l in three with small cell carcinoma, the increase in those with squamous cell carcinoma being significant. The serum PTHrP concentration was within normal limits in all patients with malignancies in the gastrointestinal system, liver, gall bladder or pancreas (Fig. 2). One patient with prostatic cancer showing osteoblastic metastasis had a serum PTHrP concentration of 242.4 pmol/l, but a normal serum calcium level. The serum PTHrP concentrations in patients with pituitary adenoma, including 11 with acromegaly, 7 with Cushing's disease, 4 with prolactinoma, 5 with nonfunctional adenoma and 4 with craniopharyngioma, were within the normal range (Fig. 2).

Changes in serum PTHrP concentrations during the clinical courses of three patients

Case 1. A 59-year-old male was diagnosed as having diffuse, large cell type, B cell non-Hodgkin's lymphoma by lymph node biopsy in June 1991. On July 31, the patient became somnolent and was admitted to this hospital. He was judged to be in stage IVb and found to have swelling of the cervical and axillary lymph nodes, jaundice, hepatomegaly, ascites and hypercalcemia (3.25 mmol/l). His serum and urinary PTHrP levels were markedly elevated (Fig. 3). Anti-HTLV-I antibody titers were negative. After chemotherapy with MACOP-B, the lymphadenopathy, jaundice, hepatomegaly and ascites disappeared, his serum and urinary PTHrP levels decreased and his serum calcium concentration was normalized. Thus, complete remission (CR) was obtained. However, his serum and urinary PTHrP levels began to increase from November and axillary lymph node swelling reappeared in December. Chemotherapy with POEM resulted in slight decreases in the size of the lymph nodes and the serum PTHrP concentration.

The malignant cells in the formalin-fixed, paraffin-embedded section of a lymph node showed positive immunoreactivity for two types of antibody, one raised against PTHrP (34–53) (Oncogene Science, NY) (Fig. 4), and the other against PTHrP (109–141) (Daiichi Radioisotope Institute, Tokyo, Japan). These large tumor cells were also stained with anti-B cell antibody L26, but not with anti-T cell antibody UCHL-1, indicating that they were B cells. This lymph node was obtained at biopsy in June 1991.

Case 2. A 68-year-old male was diagnosed as having diffuse, large cell type, B cell non-Hodgkin's lymphoma by lymph node biopsy in March, 1991. When admitted to our hospital in September, he was judged to be in stage IVb and showed disturbance of consciousness, swelling of the cervical, axillary and inguinal lymph nodes and hypercalcemia (3.40 mmol/l). Anti-HTLV-I antibody titers were negative. His serum PTHrP concentration was 461.5 pmol/l. After chemotherapy with MACOP-B, the lymphadenopathy subsided and the serum PTHrP concentration decreased to 34.1 pmol/l and his serum calcium concentration (2.27 mmol/l) was normalized. However, the patient died of sepsis on 9 October.

Case 3. A 54-year-old male with cheek swelling was diagnosed as having squamous cell carcinoma of the maxillary sinus in August, 1988. His serum PTHrP concentration was slightly elevated to 64.0 pmol/l, but the serum calcium level was normal (Fig. 5). After operation, he received chemotherapy and radiation therapy, resulting in decrease of the tumor size and of the serum PTHrP concentration in June, 1989. However, subsequently his serum PTHrP concentration increased almost in parallel with enlargement of the tumor and the serum squamous cell carcinoma-related antigen (SCC) level. Hypercalcemia developed in December, 1990 and he died of respiratory failure due to multiple metastasis in the lung.

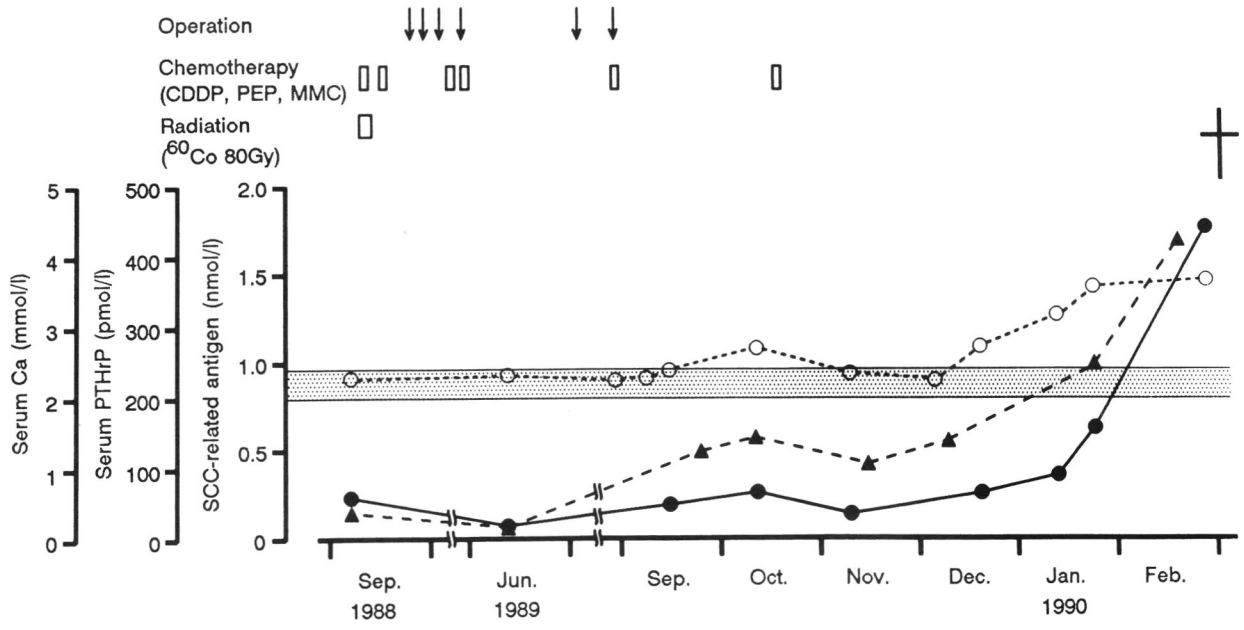

Fig. 5. Clinical course of Case 3. The serum levels of PTHrP (●), squamous cell carcinoma-related antigen (SCC) (▲) and calcium (Ca) (○) are shown. The shaded area represents the normal range of serum calcium level. CDDP: cis-diamine dichloroplatinum. PEP: peplomycin. MMC: mitomycin C.

Discussion

Burt et al. (11) reported that the incidence of hypercalcemia in patients with malignancy is about 8.5%, and is similar in patients with solid tumors and hematologic malignancies. There are also reports of association of hypercalcemia with hematologic malignancy in about 30% of myeloma patients, 40% of adult T cell leukemia patients, 1.3% of leukemia patients, and 5.6% (12) or 1.8% (13) of malignant lymphoma patients.

PTHrP has been implicated as the main causal factor of humoral hypercalcemia of malignancy. Since the determination of its amino acid seqeunce by Suva et al. (5) in 1987, several methods for its determination have been developed for study of the mode of its production and secretion.

Budayr et al. (14) developed an RIA for PTHrP (1–34) in 1989, and reported high serum PTHrP (1–34) concentrations in 30 (71%) of 42 patients with solid tumors associated with hypercalcemia. Most of these tumors were squamous cell carcinomas and renal cancers. They also found that 23 patients with normocalcemia nearly all had a normal serum PTHrP concentration. Similarly, Kao et al. (15) observed elevated plasma PTHrP (1–34) concentrations in 47% of patients with cancer associated with hypercalcemia. In 1990, Burtis et al. (8) developed an IRMA for PTHrP (1–74) and an RIA for PTHrP (109–138), and reported that the plasma PTHrP concentration was markedly increased in humoral hypercalcemia of malignancy patients, but not in cancer patients with normocalcemia or local osteolytic hypercalcemia patients, and that the plasma PTHrP concentration was low or undetectable in 60 normal subjects. Therefore, he suggested that determination of the plasma PTHrP concentration was useful for diagnosis of humoral hypercalcemia of malignancy. Henderson et al. (16) reported similar findings. However, it is difficult to measure the serum PTHrP concentrations in normal subjects or patients with tumors with normocalcemia by these assays, and so there have been few reports on the changes in serum PTHrP levels in patients during their clinical course.

In this study, we could measure serum PTHrP concentrations in normal subjects and patients by an RIA for PTHrP (109–141) developed by Kasahara et al. (10). This RIA can detect as little as 2 pmol/l of PTHrP, and its specificity and reproducibility are excellent. We found that the serum PTHrP concentrations in 167 normal subjects were 20.2–50.8 pmol/l. Seven of 56 patients with haematologic malignancy had hypercalcemia, and 4 of them (2 with malignant lymphoma, 1 with adult T cell leukemia and 1 with myeloma) had very high serum PTHrP concentrations. These data are consistent with reported findings (14, 17, 18). Moreover, our series included a patient with B cell non-Hodgkin's lymphoma in whom the serum PTHrP concentration was elevated and production of PTHrP by the lymphoma cells was demonstrated immunohistochemically. This seems to be

the first report of a PTHrP-producing B cell type lymphoma.

There are reports of PTHrP production in a number of malignant cell lines and in solid tumors causing humoral hypercalcemia of malignancy. In this study, 8 of 144 patients with solid tumors had hypercalcemia and markedly high serum PTHrP (109–141) concentrations. These findings confirm the conclusion that determination of the serum PTHrP (109–141) is useful for diagnosis of humoral hypercalcemia of malignancy. Increased serum PTHrP concentrations were found in 80% of the patients with malignancy with hypercalcemia, indicating that most of these malignancies with hypercalcemia were due to PTHrP production. In the other cases, several other factors may have been involved, such as interleukin-1 (19), osteoclast activating factor (20), prostaglandins (21) and transforming growth factor (22).

The other 136 patients with solid tumors had normocalcemia, but the serum PTHrP concentration was slightly elevated in seven patients (three with oral squamous cell carcinoma, three with lung squamous cell carcinoma and one with prostatic cancer). All these seven patients were advanced cases at clinical stage III or IV. Only one of these seven patients later became hypercalcemic with enlargement of the tumor. These findings may be explained by the differences in the duration of elevated PTHrP levels and in the capacity of calcium excretion in these patients. Of 20 patients with lung cancer, the serum PTHrP concentration was significantly higher in those with squamous cell carcinoma than in those with adenocarcinoma or small cell carcinoma. These findings are consistent with reports that PTHrP was released into the culture medium of human keratinocytes (23), and that well differentiated squamous cell carcinomas were stained with anti-PTHrP antibody but adenocarcinomas were not irrespective of the serum calcium level (24).

Prostatic cancer is rarely associated with hypercalcemia because it is liable to show osteoblastic metastasis. Raskin et al. (25) reported that more than 30% of patients with prostatic cancer with bone metastasis had hypocalcemia, and that no cases had hypercalcemia. In this study, one patient with osteoblastic metastasis had a high serum PTHrP concentration, but a normal serum calcium level. These findings may reflect the balance between osteogenesis due to osteoblastic metastasis and bone resorption due to PTHrP.

An interesting finding was that in a patient with oral squamous cell carcinoma with normocalcemia, the serum PTHrP level began to increase in parallel with enlargement of the tumor but before increase in the serum calcium concentration. This finding shows that determination of serum PTHrP is also useful for predicting development of humoral hypercalcemia of malignancy, and that the serum PTHrP concentration can be used as one of the tumor markers.

Acknowledgments. We are grateful to the Daiichi Radioisotope Institute for supplying the PTHrP RIA kit, and to Dr T Sano, First Department of Pathology, School of Medicine, University of Tokushima, for carrying out the immunohistochemical studies.

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Received February 27th, 1992
Accepted June 15th, 1992