Lung Cancer Associated With Hypercalcemia Induced by Concurrently Elevated Parathyroid Hormone and Parathyroid Hormone-Related Protein Levels

Keiko Uchimura, Takehiko Mokuno, Akio Nagasaka, Nobuki Hayakawa, Taiya Kato, Naho Yamazaki, Takako Kobayashi, Mutsuko Nagata, Motoko Kotake, Mitsuyasu Itoh, Toru Tsujimura, and Katsumi Iwase

In general, many cases of malignancy-associated hypercalcemia are due to HHM. In patients with humoral hypercalcemia of malignancy (HHM), it has been reported that plasma parathyroid hormone-related protein (PTHrP) and cyclic adenosine monophosphate (cAMP) levels were elevated, while plasma PTH and active vitamin D_3 levels were suppressed. Our patient showed hypercalcemia with a concurrent increase in plasma and tumor tissue PTHrP and PTH concentrations and also high cAMP and low 1-25$(OH)_2VD_3$ levels in the plasma. These data suggest that the hypercalcemia exhibited by our patient was consistent with HHM due to lung cancer and its liver metastasis. Moreover, diagnostic imaging and autopsy findings showed no appreciable lesions of the parathyroid gland. In addition, histopathologic examination of the primary and metastatic tumors revealed the existence of PTH immunohistochemically stained with anti-PTH antibodies, suggesting an ectopic–PTH-producing lung tumor. From these data, our patient was diagnosed with a rare case of lung cancer, which produced both ectopic PTH and PTHrP.

MALIGNANCY-ASSOCIATED hypercalcemia is a complication in a wide range of cancers, from solid cancers, such as squamous cell carcinoma, breast cancer, and hepatocellular carcinoma, to malignant hematologic tumors.

Hypercalcemia caused by malignant tumors has been conventionally classified into local osteolytic hypercalcemia (LOH) and humoral hypercalcemia of malignancy (HHM). It is believed that LOH is induced by bone destruction due to a malignant tumor, while HHM is caused by the effect of humoral factors produced by the tumor in its calcium metabolism. In 1987, Moseley et al,[1] Strewler et al,[2] and Burtis et al[3] purified a protein with parathyroid hormone (PTH)-like activity from the tissues of lung, renal cell, and breast carcinomas and the supernatants of those cultured cells, respectively. Suva et al[4] cloned a protein from cDNA, identified its amino acid sequences, and showed that its N-terminal structure was homologous to that of PTH. The protein, known as PTH-related protein (PTHrP), shows a similar affinity for PTH receptors as PTH and appears to exhibit the same physiologic activity. Various patients with HHM were analyzed, and it was clarified that PTHrP was a major causative agent of HHM.[5] Eighty percent of malignancy-associated hypercalcemia is due to HHM. However, ectopic–PTH-producing tumors are rarely the cause of hypercalcemia.

The present report describes a patient with squamous cell carcinoma of the lung who was admitted to our university hospital as an emergency because of a remarkably high level of plasma calcium induced by concurrently elevated PTH and PTHrP levels. The excessive production of both ectopic PTH and PTHrP in the lung tumor tissue was also confirmed by autopsy examination of the patient.

CASE REPORT

The patient was a 74-year-old man complaining mainly of disturbed consciousness. His family history was unrelated. Since 1982, he has suffered from chronic rheumatoid arthritis.

The patient has suffered from anorexia and general fatigue and tended to be somnolent for about 1 year. Therefore, he was examined by a local medical doctor and referred to the emergency clinic of our university hospital because his laboratory tests revealed a calcium (Ca) level of 17.6 mg/dL and his chest x-ray showed a pulmonary tumor shadow.

Physical examination revealed as follows: body height, 156 cm; body weight, 45.3 kg; blood pressure, 150/70 mm Hg; pulse rate, 84/minute, regular; and consciousness, unclear. Neither jaundice nor anemia were observed in the conjunctiva. His respiratory sounds were clear, and no heart murmur was audible. The liver was palpable across a 3-finger breadth below the right costal margin in the right upper abdominal quadrant. There was no pretibial pitting edema or anasarca. The patient did not complain of ostealgia.

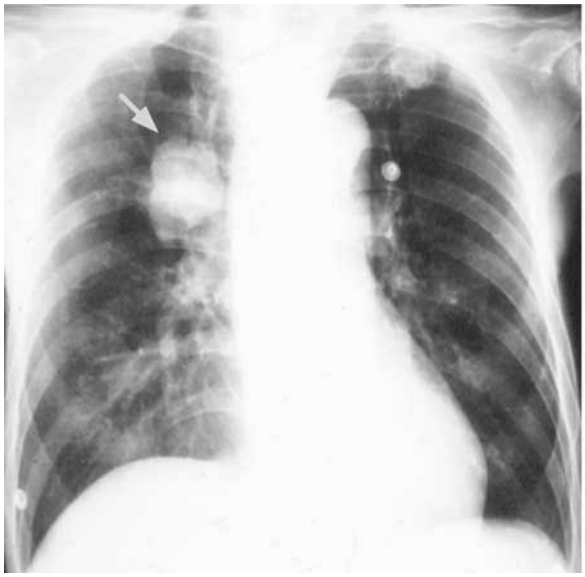
Laboratory tests on admission: anemia, liver dysfunction, and relatively mild renal dysfunction (blood urea nitrogen [BUN], 28 mg/dL; creatinine, 1.3 mg/dL) with preservation of urine volume were observed. Serum electrolytes showed a high Ca level (16.6 mg/dL [verified by serum albumin concentration], ionized Ca, 7.41 mg/dL). Urinary Ca excretion was also high (Ca, 476 mg/d [normal value, 50 to 300], urinary protein, 256 mg/d [normal value, 80 to 150], urinary creatinine, 256 mg/d [normal value, 70 to 160]). Mild metabolic alkalosis was observed on blood gas analysis with room air (pH 7.449; P_{CO_2}, 43.1 mm Hg; P_{O_2}, 78.6 mm Hg; base excess (BE), 6.1 mmol/L; bicarbonate [HCO_3], 30.3 mmol/L; oxygen saturation, 95.7%). A chest x-ray revealed a tumor shadow 6 cm in diameter with an irregular margin at the right hilus. Computed tomography (CT) scanning of the chest showed mediastinal infiltration by the tumor in the right lung lobe region (Fig 1), but the other region showed no bone metastatic lesion. Endocrinologic in-

From the Division of Endocrinology and Metabolism, Departments of Internal Medicine and Surgery, Fujita Health University School of Medicine, Toyoake, Aichi, Japan.

Submitted October 6, 2001; accepted January 22, 2002.

Address reprint requests to Mitsuyasu Itoh, MD, Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan.

0026-0495/02/5107-0005$35.00/0
doi:10.1053/meta.2002.33341



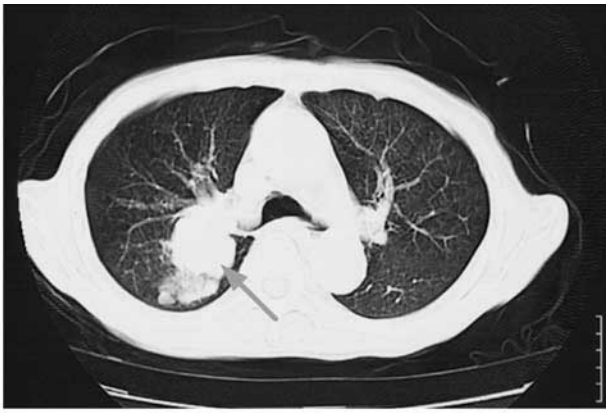


Fig 1. Chest x-ray revealed a tumor shadow (arrow) 6 cm in diameter with an irregular margin at the right hilus. CT scanning of the same area of the chest showed mediastinal infiltration of the tumor in the right lung lobe region.

vestigations (Table 1) showed the high plasma PTHrP level with a concurrent increase in the plasma C-terminal, sensitive and intact PTHs and serum cyclic adenosine monophosphate (cAMP), and a decrease in the plasma 1-25$(OH)_2VD_3$ level. The other endocrinologic tests were normal. The assay methods used for each hormone are shown in Table 1. Ultrasonography on his neck showed no abnormal shadow in the thyroid or parathyroid glands. Abdominal ultrasonography revealed no hepatic tumors or other abnormalities.

Table 1. Endocrinologic Findings

Serum TSH	3.2 μU/mL	(0.4-3.5)
Serum T_3	0.7 ng/mL	(0.7-1.6)
Serum T_4	11.3 μg/dL	(5.4-10.5)
Serum PTH (C-terminal)	2.8 ng/mL	(0.5>)
Serum PTH (sensitive)	3,600 pg/mL	(160-520)
Serum PTH (intact)	180 pg/mL	(15-50)
Plasma PTHrP	3.09 pmol/L	(0.3-0.75)
Serum calcitonin	51 pg/mL	(100>)
Serum 1-25 $(OH)_2$ VD_3	5.0> pg/mL	(20-60)
Serum cAMP	41 pmol/mL	(11-21)
Urinary cAMP	0.01> μmol/d	(1.8-6.3)
Plasma ACTH	11 pg/mL	(9-52)
Serum cortisol	22 μg/dL	(5.3-24.5)

NOTE. Numbers in parentheses are the normal values. Intact PTH concentration was measured with a 2-site chemiluminescent immunoassay from Nichols Institute Diagnostics (San Juan Capistrano, CA) (minimal detectable value, 2 pg/mL; intra-assay CV, 4.6%; interassay CV, 3.9%). PTHrP concentration was measured with a 2-site immunoradiometric assay from Nichols Institute Diagnostics (minimal detectable value, 0.3 pmol/L; intra-assay CV, 2.5%; interassay CV, 7.6%). Calcitonin (minimal detectable value, 10 pg/mL; intra-assay CV, 4.5%; interassay CV, 4.4%; 1 25 $(OH)_2$ VD_3 (2 pg/mL, 8.8%, 8.0%); cAMP (0.62 pmol/mL, 4.2%, 4.9%); ACTH (5 pg/mL, 2.5%, 3.6%); cortisol (1 μg/dL, 4.9%, 7.1%); C-terminal (0.2 ng/mL, 8.4%, 9.9%) and sensitive PTH (100 pg/mL, 6.2%, 4.1%) concentrations were measured with commercially available radioimmunoassay kits. TSH, T_3, and T_4 concentrations were measured with a chemiluminescent enzyme immunoassay. These PTHs and PTHrP assays showed no cross-reactivity with each other.

Abbreviations: TSH, thyroid-stimulating hormone, T_3, triiodothyronine; T_4, thyroxine; ACTH, corticotropin.

Concerning his progress, serum Ca level normalized after he was treated with electrolyte infusion, diuretics, calcitonin, and pamidronate disodium. Hemodialysis was also applied to reduce his high Ca level (Fig 2). However, the patient died of hepatic failure complicated with disseminated intravascular coagulation 21 days after admission.

The autopsy examination revealed a 6-cm lung carcinoma in the upper right lobe with tumor metastases in the liver and both lungs. Hemorrhagic ascites and pleural fluids were also observed. Hepatic tumor invasion was observed. The thyroid and parathyroid glands were normal size and weight. The histopathologic examination for these glands also confirmed that the parathyroid glands were normal, and no ectopic parathyroid glands were found in the mediastinum. Histologic examination of the lung tumor mass (Fig 3A) showed a solid lung tumor accompanied by extensive necrosis. The tumor was characterized by proliferation of atypical cells with a cobblestone-like and, in parts, onion-like arrangement. The diagnosis was moderately well-differentiated squamous cell carcinoma with high mitotic nuclei. The histologic findings of the liver tumor (Fig 3B) were similar to those in the lung, suggesting hematogenous and lymphatic metastases of a moderately well-differentiated squamous cell carcinoma of the lung. In the tumor, relatively well-differentiated and spindle-shaped tumor cells were stained positively with PTH antibodies, which did not cross-react with PTHrP (Fig 4A), compared with negative control staining (Fig 4B), and electron microscopy (Fig 5) showed scattered secretory granules inside the tumor cells. PTH and PTHrP concentrations concurrently increased in the lung and liver tumor tissues (the methods for PTH and PTHrP measurement in the lung and liver tissues are shown in the legend to Table 2) as did those in the plasma. We tried to confirm PTH and PTHrP mRNAs using a cDNA probe prepared by Dr H. Katagami,

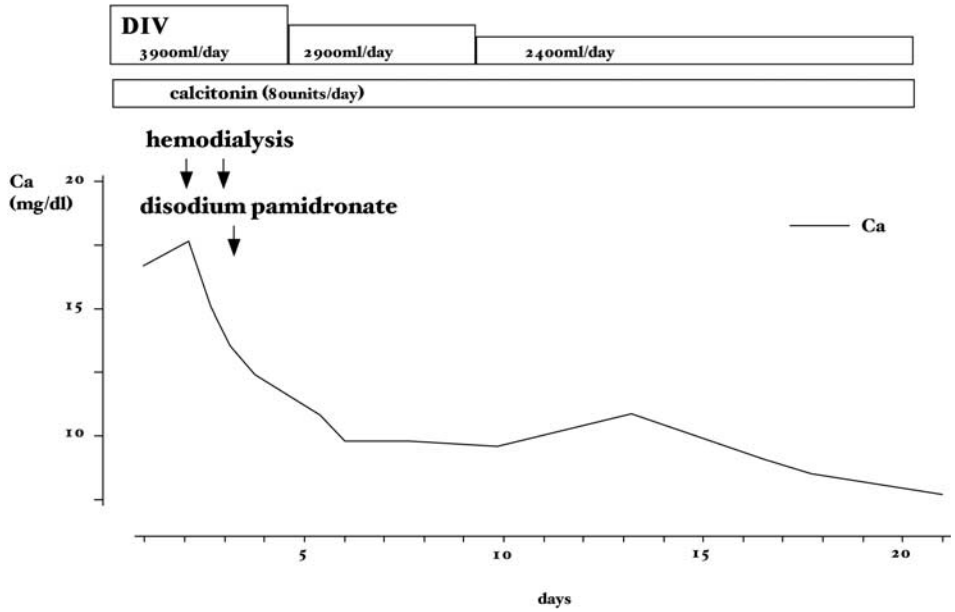


Fig 2. Changes in serum Ca levels during therapy.

Miyazaki Medical College, Miyazaki, Japan, and PTHrP staining was tried using a PTHrP antibody prepared by Dr H. Katagami and a PTHrP monocronal antibody from Oncogene Science, Cambridge, MA. However, as PTHrP is unstable, this was unsuccessful probably because the specimens were obtained from the patient at autopsy.

DISCUSSION

In patients with HHM, it was reported that plasma PTHrP and cAMP levels were elevated, while plasma PTH and active vitamin D_3 levels were suppressed. In HHM patients, the concept of ectopic–PTH-producing tumors was suggented, but this idea has been rejected because PTHrP has been identified. Cases of malignant tumors associated with high plasma PTH levels should be suspected as malignant tumors complicated with primary hyperparathyroidism. The diagnosis of an ectopic–PTH-producing tumor should be made only after primary or secondary hyperparathyroidism can be ruled out.

Our patient showed hypercalcemia with a concurrent increase in plasma and tumor tissue PTHrP and PTH concentrations and also high cAMP and low 1-25$(OH)_2VD_3$ levels in the plasma. Whether or not the low urinary cAMP level was due to a defect in the nephrogenic action of PTH and PHTrP or hypercalcemia treatment-induced renal dysfunction is unclear, although these data suggest that our patient's hypercalcemia was consistent with HHM due to lung cancer and its liver metastasis. Therefore, the cause of the elevated plasma PTH level was thoroughly investigated to rule out hyperparathyroidism. Diagnostic imaging showed no appreciable lesions of the parathyroid gland, which was confirmed at autopsy. In addition,

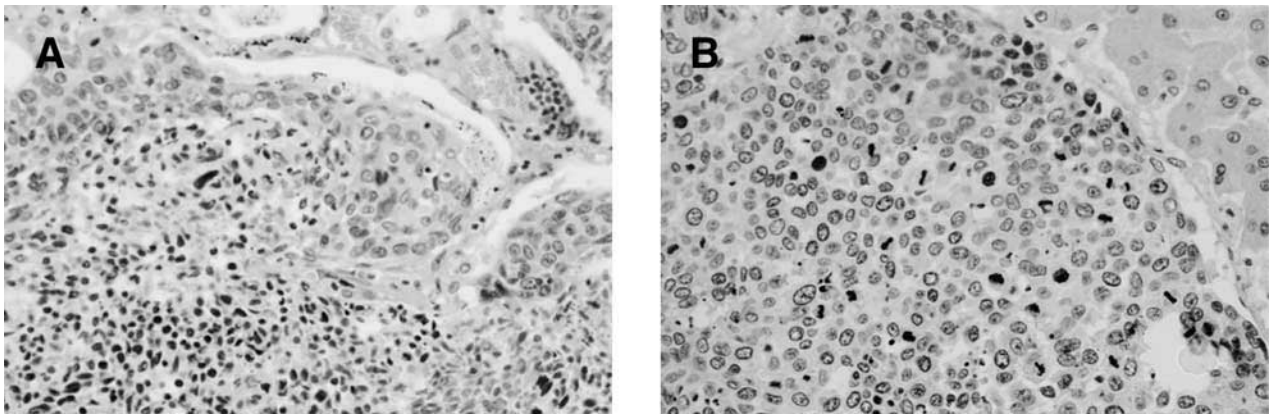


Fig 3. (A) Histologic findings of the lung tumor stained with hematoxylin-eosin (original magnification × 200). The tumor, characterized by proliferation of atypical cells with a cobblestone-like and, in parts, onion-like arrangement, was diagnosed as a moderately well-differentiated squamous cell carcinoma with high mitotic nuclei. (B) The histologic findings of the metastatic tumor in the liver stained with hematoxylin-eosin (original magnification x 200). Tumor cells are similar to those in the lung, suggesting hematogenous and lymphatic metastases of a moderately well-differentiated squamous cell carcinoma of the lung.

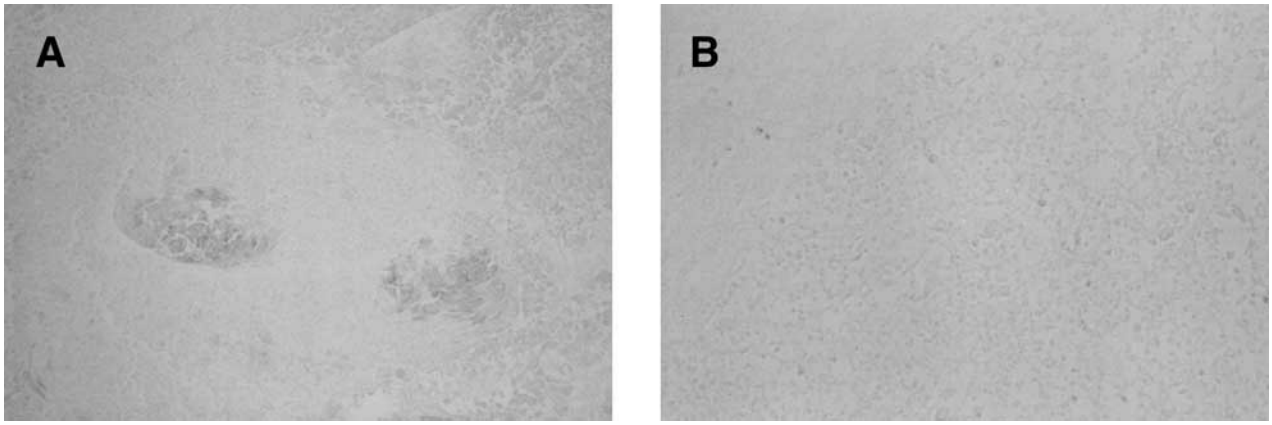


Fig 4. (A) Immunostaining of the lung tumor with PTH antibodies (original magnification × 200). In the tumor, relatively well-differentiated and spindle-shaped tumor cells are stained positively with PTH antibodies from Bio-mega Co, Ltd, Sostercity, CA. (B) Negative control staining did not show any visible staining. The cross-reactivity of the antibody used for PTH with PTHrP was lacking.

histopathologic examination of the primary and metastatic tumors also revealed the existence of PTH immunohistochemically stained with anti-PTH antibodies, suggesting an ectopic–PTH-producing lung tumor. There have been many reports of ectopic–PTH-producing tumors since 1987, including small cell carcinoma of the lung by Yoshimoto et al,[6] primitive neuroectodermal tumor by Strewler et al,[7] ovarian cancer by Nussbaum et al,[8] cholangiocellular carcinoma by Baba et al,[9] and thymoma by Rizzoli et al.[10] The tumor in our patient was histologically identified as a squamous cell carcinoma and was neuron-specific enolase (NSE)-positive. The presence of secretory granules inside the tumor cells was confirmed by electron microscopy. The tumors in the patients of Yoshimoto et al[6] and Strewler et al[7] were histologically characterized by positive NSE staining, but their tissue PTH production was unclear.

PTHrP consists of 141 amino acids and its actions on the mid- and C-terminal are largely unknown. However, the N-terminal structure is homologous to that of PTH, which consists of 84 amino acids. Injection of PTHrP N-terminal fragments into rats resulted in an increase in 1-25$(OH)_2VD_3$[11] unlike HHM, and the PTHrP C-terminal inhibited osteoclasts.[12] These findings indicate that the pathophysiologic changes in HHM cannot be explained by the PTH-like activity of PTHrP alone. In the patient reported by Strewler et al,[7] the blood PTHrP concentration was slightly elevated, and the presence of PTHrP mRNA in the tumor tissue was confirmed. They assessed the tumor's correlation with hypercalcemia, but did not discuss the change in the plasma 1-25$(OH)_2VD_3$ level.

The normal circulation level of PHTrP is considerably lower than the level of PTH. Therefore, it is uncertain that PTHrP has some role in maintaining calcium homeostasis. However, PTHrP is widely present in fetal tissues, and its gene is expressed in developing and adult tissues, suggesting it has some

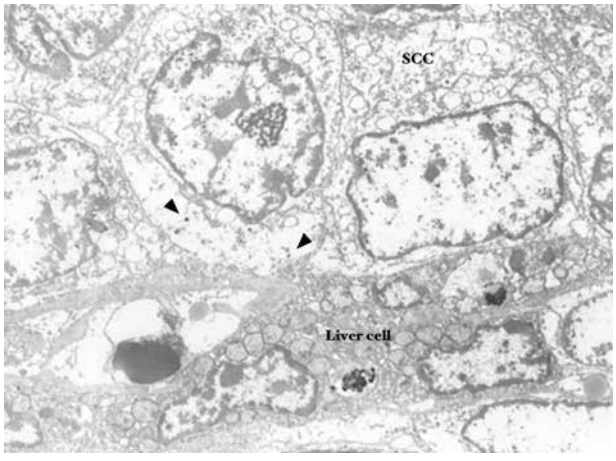


Fig 5. Electron microscopic findings of the lung metastatic tumor in the liver (original magnification × 3,900). Scattered secretory granules (arrow) are observed inside the tumor cells (squamous cell carcinoma [SCC]).

Table 2. PTH and PTHrP Concentrations in the Lung and Liver Tumor Tissues of the Patient

		PTH		PTHrP
Lung	C-terminal	12.67	ng/g wet tissue	
	sensitive	124,171	pg/g wet tissue	0.73 pmol/g wet tissue
	intact	25>	pg/g wet tissue	
Liver	C-terminal	20.83	ng/g wet tissue	
	sensitive	23,449	pg/g wet tissue	0.13 pmol/g wet tissue
	intact	4,177	pg/g wet tissue	

NOTE. The tissues were homogenized in an 8 mol/L urea buffer containing 0.2 N HCl for the tissue PTH assay, and PTH was extracted according to the method reported previously.[14] For the tissue PTHrP assay, the tissues were homogenized in 1.0 mol/L acetic acid, and PTHrP was extracted according to the previously reported method.[15] The minimal detectable values were 0.1 ng/g (wet weight tissue) in c-terminal PTH, 50 pg/g in sensitive PTH, and 25 pg/g in intact PTH in lung and liver tissues, while that of PTHrP was 0.03 pmol/g in lung and liver tissues. PTH or PTHrP antibody did not cross-react with each other. C-terminal, sensitive, and intact PTHs were undetectable in normal lung and liver tissues. PTHrP was also undetectable in normal lung and liver tissues.

functions in development and in normal physiology at the cell or tissue level.

Further studies on developmental expression of PTH and PTHrP linked to gene sequence, such as Southern and Western blotting analyses,[13] may allow us to draw the inference of gene function as an underlying cause of PTH and PTHrP production by the tumor in this patient.

Our patient had a rare case of lung cancer, which produced both ectopic PTH and PTHrP, of which the pathophysiologic changes are of particular interest when considering HHM.

ACKNOWLEDGMENT

We thank Dr Hideyoshi Katagami, Miyazaki Medical College, Miyazaki, Japan, for providing PTHrP-antibody and its cDNA probe and for offering suggestions about our study. We also thank N. Takekawa for preparing the manuscript.

REFERENCES

1. Moseley JM, Kubota M, Diefenbach-Jagger H, et al: Parathyroid hormone-related protein purified from a human lung cancer cell line. Proc Natl Acad Sci USA 84:5048-5052, 1987

2. Strewler GJ, Stern PH, Jacobs JW, et al: Parathyroid hormone-like protein from human renal carcinoma cells. Structural and functional homology with parathyroid hormone. J Clin Invest 80:1803-1807, 1987

3. Burtis WJ, Wu T, Bunch C, et al: Identification of a novel 17,000-dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. J Biol Chem 262:7151-7156, 1987

4. Suva LJ, Winslow GA, Wettenhall RE, et al: A parathyroid hormone-related protein implicated in malignant hypercalcemia: Cloning and expression. Science 237:893-896, 1987

5. Ikeda K, Mangin M, Dreyer BE, et al: Identification of transcripts encoding a parathyroid hormone-like peptide in messenger RNAs from a variety of human and animal tumors associated with humoral hypercalcemia of malignancy. J Clin Invest 81:2010-2014, 1988

6. Yoshimoto K, Yamasaki R, Sakai H, et al: Ectopic production of parathyroid hormone by small cell lung cancer in a patient with hypercalcemia. J Clin Endocrinol Metab 68:976-981, 1989

7. Strewler GJ, Budayr AA, Clark OH, et al: Production of parathyroid hormone by a malignant nonparathyroid tumor in a hypercalcemic patient. J Clin Endocrinol Metab 76:1373-1375, 1993

8. Nussbaum SR, Gaz RD, Arnold A: Hypercalcemia and ectopic secretion of parathyroid hormone by an ovarian carcinoma with rearrangement of the gene for parathyroid hormone. N Engl J Med 323: 1324-1328, 1990

9. Baba H, Kawaminami T, Watanabe S, et al: The first case of bile duct adenocarcinoma producing PTH confirmed using immunohistochemical analysis and in situ hybridization. Folia Endocrinol Jpn 69: 438, 1993 (abstr)

10. Rizzoli R, Pache JC, Didierjean L, et al: A thymoma as a cause of true ectopic hyperparathyroidism. J Clin Endocrinol Metab 79:912-915, 1994

11. Horiuchi N, Caulfield MP, Fisher JE, et al: Similarity of synthetic peptide from human tumor to parathyroid hormone in vivo and in vitro. Science 238:1566-1568, 1987

12. Fenton AJ, Kemp BE, Hammonds RG Jr, et al: A potent inhibitor of osteoclastic bone resorption within a highly conserved pentapeptide region of parathyroid hormone-related protein; PTHrP. Endocrinology 129:3424-3426 1991

13. Arnold C, Metherell L, Clewley JP, et al: Predictive modeling of fluorescent AFLP: A new approach to the molecular epidemiology of E. coli. Res Microbiol 150:33-44 1999

14. Kemper B, Habener JF, Potts JT, et al: Proparathyroid hormone: Identification of a biosynthetic precursor to parathyroid hormone. Proc Natl Acad Sci USA 69:643-647, 1972

15. Docherty HM, Ratcliffe WA, Heath DA, et al: Expression of parathyroid hormone-related protein in abnormal human parathyroids. J Endocrinol 129:431-438, 1991