PTHrP-producing Tumor: Squamous Cell Carcinoma of the Liver Accompanied by Humoral Hypercalcemia of Malignancy, Increased IL-6 and Leukocytosis

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Abstract

A 77-year-old man was admitted to our hospital showing symptoms of general fatigue and appetite loss. He had leukocytosis, thrombocytosis and hypercalcemia with elevated serum levels of parathyroid hormone related peptide (PTHrP) and interleukin-6 (IL-6). An increase in tumor markers SCC and CYFURA21-1 was observed. The liver contained a huge tumor, which was proved to be PTHrP producing squamous cell carcinoma by immuno-histochemical analysis. Since the tumor did not express IL-6, it was assumed to be induced by PTHrP in osteoblasts. This is the first report of PTHrP producing squamous cell carcinoma of the liver.

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Key words: calcium, parathyroid hormone, cytokine, liver cyst

Introduction

Parathyroid hormone related peptides (PTHrP) were first identified in 1987. The physiological function of PTHrP is thought to be a local growth factor, but it has not been analyzed closely. Serum PTHrP level in the body is low and not detectable in the physiological state. When cancer cells produce a large amount of PTHrP, serum PTHrP level is elevated. PTHrP then binds to the PTH/PTHrP receptors in both the bone and kidney, which induces intracellular signal transduction, resulting in serum calcium increase. These symptoms are called humoral hypercalcemia of malignancy (HHM) (1, 2). Among the many kinds of cancer cells, more than half of HHM is caused by squamous cell carcinomas (3) originating in the lung, skin and alimentary tract. Most of the squamous cells and squamous cell carcinoma express PTHrP (4), but elevated serum

concentrations of PTHrP can be detected only in patients with cancer that express high levels of PTHrP. Squamous cell carcinoma of the liver is a very rare disorder, which has been reported only in 18 cases to date. Among them, only one case was associated with hypercalcemia (5). In this paper, we report a case of PTHrP producing squamous cell carcinoma of the liver with HHM, high serum IL-6 level, leukocytosis and thrombocytosis.

Case Report

A 77-year-old Japanese man was admitted to our hospital in December 1997. He had suffered from general fatigue, appetite loss and body weight loss from 55 kg to 47 kg since August 1997. His symptoms had become worse during the previous two months. His family history revealed that his mother had rheumatoid arthritis, but there were no other particular diseases among his relatives. He had cervical spondylosis and had undergone surgery for bilateral cataracts at the age of 76.

On admission he was generally emaciated, 159 cm in height, weight 47 kg, and showed signs of cachexia. Blood pressure was 130/68 mmHg. Icterus and anemia were not observed in his conjunctiva. There were no pathological findings on his skin. Chest sound was vesicular and the heart had no murmurs. His abdomen was flat and no venous dilatation was observed. The liver, spleen, kidney and the tumor, as well, were not palpable.

Laboratory data showed mild anemia, leukocytosis and thrombocytosis (Table 1). Serum calcium level was 16.7 mg/ml and still high after adjustment by serum albumin level. Liver function tests were within the normal range except for a mild increase in γ glutamyl transpeptidase level. Hepatitis C virus antibody was positive, but hepatitis C virus RNA was negative. Increase in C-reactive protein and tumor markers, such as SCC, CYFURA21-1 and neuron-specific enolase (NSE) were observed. These findings suggested that the patient was carry-

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Table 1. Laboratory Data

<Complete blood cell count>		<Virus antibody>	
WBC	22.0×10 ³ /mm ³	Hepatitis C virus antibody	(+) 3.77 U
(Neutrophil 79%, Lymphocyte 15% Monocyte 5%, Eosinophil 1%)		Hepatitis C virus RNA	(-)
RBC	369×10 ⁴ /mm ³	Hepatitis B virus surface antigen	(-)
Hemoglobin	11.1 g/dl	Hepatitis B virus surface antibody	(-)
Platelet	41.9×10 ⁴ /mm ³	Hepatitis B virus core antibody	(-)
<Biochemical data>		<Tumor markers>	
Ca	16.7 mg/dl	CEA	2.9 ng/ml
P	3.3 mg/dl	AFP	3.1 ng/ml
Total-protein	7.7 g/dl	PIVKA II	35 AU/ml
Albumin	3.7 g/dl	SCC	9.2 ng/ml (≤ 1.5)
AST	24 IU/l	NSE	13 ng/ml (≤ 10)
ALT	15 IU/l	CYFURA21-1	37.2 ng/ml (≤ 3.5)
γGTP	64 IU/l	ProGRP	17 pg/ml
ALP	356 IU/l	<Hormones and cytokines>	
LDH	248 IU/l	PTHrP	333 pmol/l (13.8–55.3)
Total-bilirubin	0.55 mg/dl	PTH	<0.6 pmol/l (160–520)
ZTT	11.3 Kunkel Unit	Calcitonin	56.3 pmol/l (31.9–43.3)
Choline esterase	131 IU/l	IL-6	122.2 pg/ml (0.16–7.25)
Glucose	103 mg/dl	IL-3	<5 pg/ml
Total-cholesterol	174 mg/dl	G-CSF	14.0 pg/ml
Triglyceride	191 mg/dl	GM-CSF	3.36 pg/ml
ICG (15 min)	3%		
CRP	11.2 mg/dl		
Rheumatoid factor	(-)		
Anti-nuclear antibody	(-)		

Underlines indicate abnormal data.

ing squamous cell carcinoma in his body. Endocrinological studies showed an enhanced PTHrP level and suppressed PTH level, which resulted in hypercalcemia. Interleukin-6 (IL-6) level was also elevated. But other cytokines, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF) and interleukin-3 (IL-3) levels were not increased. These findings suggested humoral hypercalcemia of malignancy (HHM).

Chest roentgenography showed that the right diaphragm was elevated (Fig. 1A). Abdominal ultrasonography and computed tomography (CT) revealed the presence of a large tumor in S8 and S7 regions of the liver. The tumor was irregular and inhomogeneous, 11×10 cm in size, and poorly enhanced by contrast medium (Fig. 1B). Dome of the liver protruded into the right pleural cavity by mass effect of the tumor.

As there was a possibility that the tumor was metastatic, extensive examinations of skin, head, otorhinolaryngial region, chest, abdomen, and urogenital tract were performed. Gastrointestinal fibers showed evidence of atrophic gastritis but no malignant tumor was detected. Barium enema of the large intestine revealed a sigmoid colon polyp of 7 mm in diameter. Brain, chest and lower abdominal CT scans were conducted,

but no tumor was found in these regions. As bone resorption by the malignant tumor may cause hypercalcemia, systemic bone scintigraphy was performed to survey bone metastatic lesions with no remarkable findings. An otolaryngologist and a urologist examined the patient, but there was no malignant tumor. On the basis of these results, we concluded that the tumor originated in the liver.

On December 3, the patient was diagnosed with humoral hypercalcemia of malignancy. Neither surgical resection of the tumor, nor specific chemotherapy was performed, because his general condition was not good. Palliative care to reduce the serum calcium level was initiated. We first prescribed administration of intramuscular calcitonin, but this drug was not effective. From December 22, intravenous injection of bisphosphonate was performed, and the serum calcium level decreased immediately and was controlled around 10 mg/ml (Fig. 2, lower). Serum PTHrP concentration gradually increased and reached more than 600 pmol/l, which was in parallel with the elevation of the serum CYFURA21-1 level (Fig. 2, middle). These findings suggested that the tumor produced PTHrP and the gradual increase in serum PTHrP concentration had been the result of tumor growth. On the other hand, the serum CRP

Figure 1. Chest X-ray film and abdominal CT. (A) Plain chest X-ray film. The right diaphragm was elevated by the liver tumor. (B) Contrast-enhanced abdominal computed tomography. The large tumor showed low density and a well-circumscribed lesion. It was a solitary tumor on contrast-enhanced computed tomography.

Figure 2. Clinical course. The time courses of WBC, CRP, PTHrP, CYFURA21-1, and Ca are shown. The time courses of the anti-hypercalcemic therapies using calcitonin and incadronate disodium are indicated at the bottom. PTHrP: parathyroid hormone related peptide, Ca: calcium, DIC: disseminated intravascular coagulation.

concentration remained at a high level despite the fact that no apparent inflammatory disorder could be detected throughout the clinical course (Fig. 2, upper). On January 15, the patient was diagnosed with disseminated intravascular coagulation and

died on February 8 because of multiple organ failure. Needle necropsy of the tumor tissue was performed.

Immunohistochemistry of the tumor tissue was conducted. The tumor was positive for keratin (Fig. 3B), and was diag-

A

B

C

D

Figure 3. Histological analysis of the tumor tissue. Hematoxylin-eosin staining of the necropsied tumor tissue (A, $\times 400$). The samples were immunostained with anti-keratin antibody (B, $\times 400$), anti-PTHrP antibody (C, $\times 400$) and anti-IL-6 antibody (D, $\times 400$). The tumor was squamous cell carcinoma and expressed PTHrP, but did not express IL-6.

nosed with squamous cell carcinoma with the findings of hematoxylin-eosin staining (Fig. 3A). Because serum IL-6 and PTHrP levels of the patient were elevated, we examined their expression in the tumor cells. The results showed that the tumor cells were positive for PTHrP (Fig. 3C), but did not express IL-6 (Fig. 3D), while alpha-feto protein and G-CSF were not detected by immuno-histochemical analysis of the tumor tissue (data not shown).

Discussion

Here, we present a case of humoral hypercalcemia of malignancy caused by squamous cell carcinoma of the liver. The incidence rate of this tumor is reported to be about 0.005% of total autopsies (6). As seen in Table 2, there are only 18 cases, which have been reported in the English or German language literature. This low incidence rate of liver squamous cell carcinoma suggests that the presence of squamous cells is very rare in the liver, and therefore the cancer may have originated in aberrant squamous cells. This hypothesis is supported by the fact that more than half (11/18 cases) of the reported cases are associated with liver cysts (Table 2). In the 18 reported cases, not all tumors were directly associated with cysts. However an intensive search for accompanying cysts may not have been performed in every case, or the original cyst may have become atrophic because of the tumor mass effect. In our clinic, we frequently diagnose liver cysts on ultrasonographs or computed tomographs, and it is possible that the lining cells of the cysts could transform into squamous cell carcinoma. It is hard to say conclusively, as the incidence of squamous cell carcinoma of the liver is extremely low, but some squamous cell carcinomas may have emerged from the squamous cells of the liver cysts.

In the present patient, the serum levels of PTHrP and IL-6 were increased. These two factors were biologically active, because increased serum levels of their downstream effector, calcium and C-reactive protein respectively, had also been observed. This patient also showed leukocytosis and thrombocytosis, but G-CSF, GM-CSF and IL-3 were not enhanced. We cannot explain these phenomena conclusively, but we may assume that leukocytosis and thrombocytosis are downstream effects of IL-6 elevation. The tumor cells proved strongly positive for PTHrP by immunohistochemistry. On the other hand, it was negative for IL-6 and the tumor cells did not produce this cytokine. As no apparent inflammatory disorder was seen in the patient, including his lung and liver, the elevated PTHrP level may have triggered the increase in IL-6 concentration. *In vitro*, PTHrP/PTH induced IL-6 expression in osteoblasts (7, 8), which was followed by stimulation of osteoclasts and resulted in additional bone resorption (9). IL-6 transmitted the signal from osteoblasts to osteoclasts. Those interactions were supported by the fact that, even if serum PTH levels were elevated in IL-6 knockout mice, there was no addition in osteoclast activity, and blunted osteoclast activity could be overcome by IL-6 addition (10). This fact was also supported by an overexpressing system. An IL-6 and PTHrP double producing tumor showed higher osteolytic activity than a PTHrP single producer, when the cells were transplanted in nude mouse, while an IL-6 single producing tumor showed weak osteolytic activity (11). These results suggest that IL-6 is located downstream of the PTHrP/PTH signaling pathway, even though the PTHrP/PTH pathway does not fully activate the IL-6 signaling pathway. But clinically, the relation between PTHrP and IL-6 is not so clear. One study reported a correlation between serum PTHrP levels and calcium levels, but no

Table 2. Literature Review of Squamous Cell Carcinomas of the Liver

Patient	Cyst	Metaplasia	Others	Authors	Reference
32M	—	—	Teratoma	Imai (1934)	14
30M	+	+		Irwin (1944)	15
56M	+	+		Edmondson (1958)	16
59M	—	—		Geddes (1970)	17
37M	+	+		Greenwood (1972)	18
30M	+	+	Duct metaplasia	Bloustein (1976)	19
38F	—	+		Chaudhuri (1982)	20
43M	—	—		Song (1984)	21
78M	+	+	Hepatolithiasis	Gresham (1985)	6
63M	+	+		Lynch (1988)	22
63M	—	—		Arase (1988)	5
73M	—	—	Liver cirrhosis	Clements (1990)	23
51F	+	—		Roediger (1991)	24
62F	+	—		Nieweg (1992)	25
82F	+	+	Chronic inflammation	Pliskin (1992)	26
59F	+	+		Banbury (1994)	27
74F	+	—		Weimann (1996)	28
56F	—	+		Shinagawa (1996)	29
77M	—	—	Duct metaplasia	Asanuma (2001)	Present case

correlation between IL-6 levels in patients with cancer and hypercalcemia (12). These findings suggest that the IL-6 signaling pathway is not simply regulated by PTHrP signaling alone. Bone marrow cells produce many inhibitory and stimulatory factors that affect osteoclastic activity (13). Therefore, PTHrP may once induce IL-6 expression, but downstream, the signaling pathway of IL-6 may be blocked by some factors without altering the serum IL-6 concentration. As our patient had no apparent inflammatory lesions, the elevated serum IL-6 concentration was thought to be from the effect of PTHrP on osteoblasts. The IL-6 concentration correlated well with the serum calcium level, which reflects osteoclastic activities. And these relations could be accurately analyzed when the anti-IL-6 receptor antibody was therapeutically prescribed to block this cascade.

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