J Gastroenterol 1997; 32:396–400

Journal of Gastroenterology

Case report

Gastric cancer associated with overexpression of parathyroid hormone-related peptide (PTHrP) and PTH/PTHrP receptor in relation to tumor progression

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Abstract: Parathyroid hormone-related peptide (PTHrP) is involved in cell proliferation in both neoplastic and non-neoplastic tissues. We describe an autopsy case of gastric cancer in a patient who showed serum hypercalcemia and overexpression of PTHrP and PTH/PTHrP receptor in the metastatic tumor cells. The primary gastric tumor was poorly differentiated adenocarcinoma, and multiple metastases were present in the bone, multiple visceral organs, peritoneum, and lymph nodes. PTHrP and its mRNA were detected only in the metastatic tumor cells, but not in primary gastric tumor. PTH/PTHrP receptor was also demonstrated immunohistologically in metastatic tumor cells. This case suggests that the expression of PTHrP is related to tumor progression and the poor prognosis in tumors associated with humoral hypercalcemia.

Key words: PTHrP, PTH/PTHrP receptor, gastric cancer, progression

Introduction

Parathyroid hormone-related peptide (PTHrP) was initially isolated from malignancies inducing humoral hypercalcemia.[1,2] PTHrP and the PTH/PTHrP-receptor gene are widely expressed in normal and neoplastic tissues, and function in both a paracrine and an autocrine fashion.[3,4] This peptide is also involved in regulation of cell proliferation and the cell cycle.[5] A recent report suggests that PTHrP is deeply involved in the progression of metastasizing pituitary adenoma, advanced prostatic cancer, and recurrence of colon cancer.[6–9] In gastric cancer, the reported expression of PTHrP is inconsistent and the reported frequency of PTHrP immunoreactivity varies widely among investigators.[10–12] Abdeen et al.[12] have suggested that the immunoreactivity of PTHrP in gastric cancer depends on antibodies for the N-terminal or C-terminal residue of PTHrP. Their application of a monoclonal antibody for the C-terminal residue showed more than 90% positivity in gastric adenocarcinomas without hypercalcemia. We report here a case of gastric cancer in a patient in whom PTHrP was expressed only in the metastatic tumor, suggesting that PTHrP overexpression is related to tumor progression.

Case report

A 50-year-old man was referred to National Nagasaki Central Hospital for acute renal failure due to bilateral hydronephrosis. Further examination showed metastatic tumor in the retroperitoneal lymph nodes, causing ureter obstruction, and multi-organ metastases were detected by roentgenological investigations. Cervical lymph node biopsy revealed metastatic poorly differentiated adenocarcinoma. Pertinent laboratory data on admission were: white blood cell count 10200/mm,[3] hemoglobin 10.2 g/dl, albumin 3.1 g/dl, total bilirubin 0.4 mg/dl, alkaline phosphatase 126 IU/l, BUN 98.9 mg/dl, creatinine 13.6 mg/dl, Na 139 meq/l, Cl 107 meq/l, K 5.6 meq/l, Ca 8.4 mg/dl, CA19-9 29.7 U/ml, CEA 230 μg/ml. Later, serum calcium and alkaline phosphatase levels were elevated, reaching a peaks of 16.5 mg/dl (normal, 8.2–10.1 mg/dl) and 1432 IU/l (normal 88–270 IU/l) respectively. Serum PTHrP (109–149) was significantly elevated to 994 pg/ml (normal, <30 pg/ml). In spite of a careful examination, the primary site was not detected. Six months after the metastatic tumor was detected, he died of massive gastrointestinal bleeding. An autopsy was performed, revealing that the primary tumor was

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(Received June 26, 1996; accepted Oct. 25, 1996)

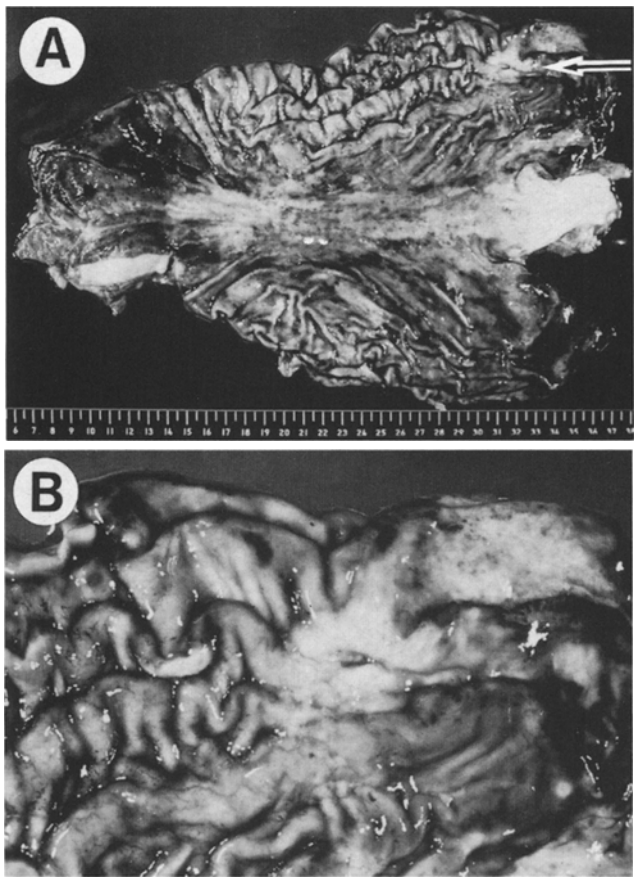

Fig. 1. **A** Borrmann type 2 gastric cancer, measuring 3 cm in diameter, in the anterior fundus (*arrow*). Histologically, poorly differentiated adenocarcinomas proliferated invasively. **B** Close view of the tumor

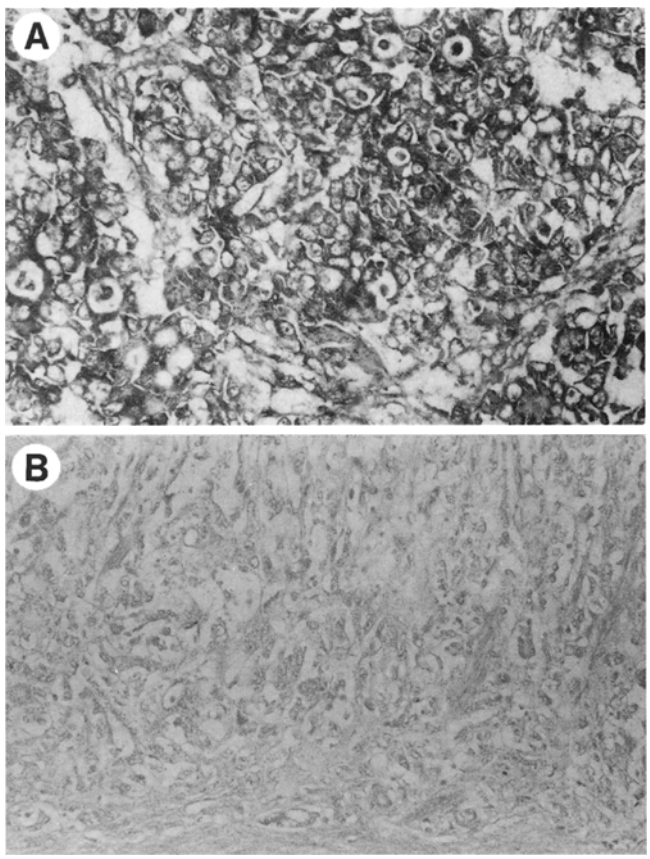

Fig. 2A,B. Immunohistochemistry revealed **A** parathyroid hormone-related peptide (PTHrP) immunoreactivity in the metastatic tumor cells in the adrenals. **B** No such immunoreactivity was detected in the tumor cells of primary gastric cancer. Immuno-alkaline phosphatase method, ×100 (**A**), ×50 (**B**)

Borrmann 2 gastric cancer, (measuring 3 cm in diameter) in the anterior fundus (Fig. 1); histologically it was poorly differentiated adenocarcinoma with minute glandular formation and mucin production. Multifocal metastases were evident in bones, kidneys, liver, lungs, adrenals, spleen, peritoneum, retroperitoneum, and lymph nodes.

All tissues were fixed in 10% neutralized formaldehyde, and deparaffinized specimens were submitted to immunohistochemical investigation and in situ hybridization. The gastric lesion was serially sectioned in four slices, 5-mm-thick, for paraffin-embedded blocks. PTHrP (38–64) immunohistochemistry was performed using monoclonal antibody at optimal dilutions (Oncogene Science, NY, USA). In situ hybridization of PTHrP mRNA was performed with digoxygenin-labeled cRNA prepared from the 343 bp PvuII/BglII fragment of the PTHrP cDNA.

PTHrP immunoreactivity was observed in the tumor cells of organs showing metastasis (Fig. 2A), including bone, visceral organs, peritoneum, and lymph nodes (Table 1). In particular, tumor cells in the involved blood vessels showed strong expression of PTHrP. By contrast, PTHrP immunoreactivity was not detected in tumor cells of the primary site (Fig. 2B). Strong expression of PTHrP mRNA was also detected in the tumor cells of metastatic sites, including bones, peritoneum,

Table 1. Expression of parathyroid hormone-related peptide (PTHrP) and PTH/PTHrP receptor in primary and metastatic tumors

Location of tumor	PTHrP	PTH/PTHrP receptor
Primary site (stomach)	−	+[a]
Metastatic sites		
Visceral organs	++/+[b]	+
Peritoneum	++	+
Retroperitoneum	++	+
Bone	++	+
Lymph nodes	++	+

−, Negative; +, weakly positive; ++, strongly positive
[a] Focally positive
[b] ++ in kidney, adrenal, liver, and spleen; + in lung

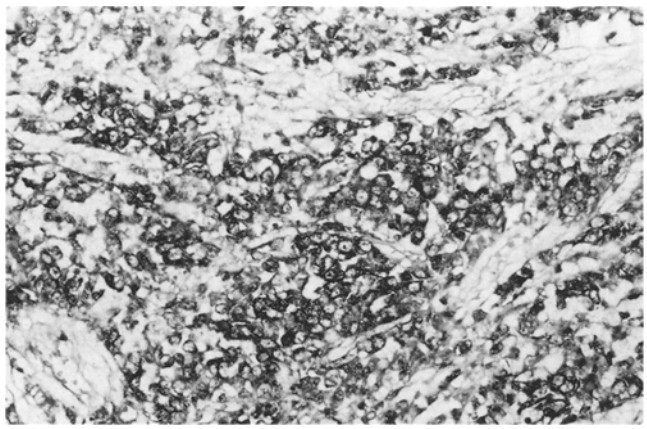

Fig. 3. In situ hybridization of PTHrP mRNA. Intense gene expression was detected in the metastatic tumor cells in the kidney. ×66

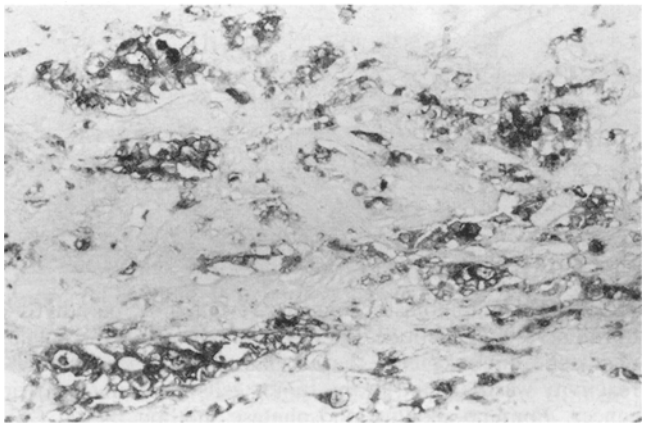

Fig. 4. Immunoreactivity for PTH/PTHrP receptor was demonstrated in the tumor cells that had invaded stroma and vessels in the stomach. ABC method, ×50

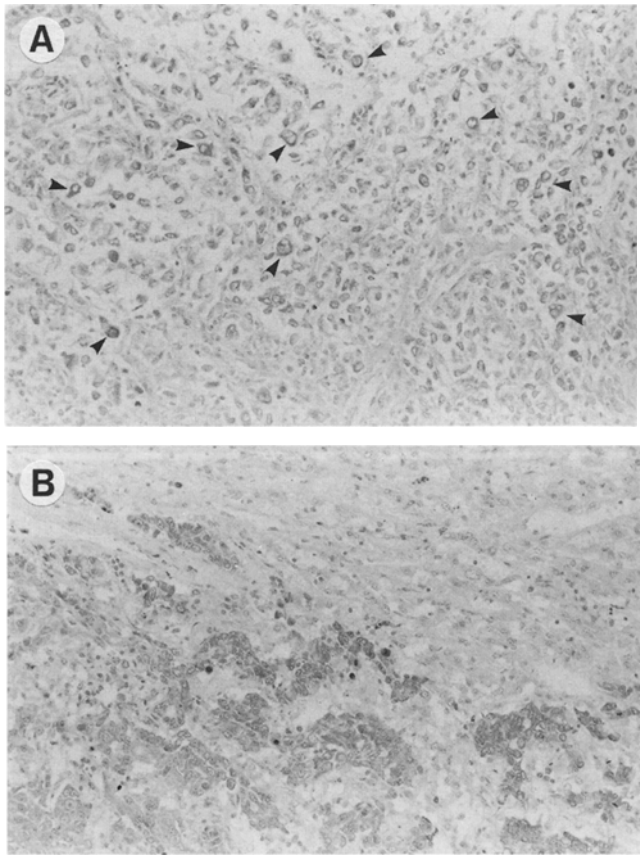

Fig. 5A,B. Immunohistochemistry of nm23. There was no apparent difference in the immunoreactivity of nm23 between **A** primary gastric tumor cells (*arrowheads*) and **B** metastatic tumor cells in the adrenals. ABC method, ×50

lymph nodes, and kidney (Fig. 3), while PTHrP mRNA was not detected in the cells of the primary tumor. Expression of PTH/PTHrP receptor (polyclonal antibody, Babco, CA, USA) was widespread in tumor cells in metastatic sites (Table 1). In the primary site, focal immunoreactivity was demonstrated in tumor cells that had deeply invaded the stroma; weak immunoreactivity was shown in vessel invasion nests (Fig. 4). In the background normal tissues, vascular and visceral smooth muscle cells showed faint expression of PTHrP and PTH/PTHrP receptor. Immunoreactivity for nm23 (H1 + H2, a monoclonal antibody, kindly provided by Dr. Furukawa, Department of Oncology, Nagasaki University) was weak in both the primary and metastatic sites (Fig. 5). p53 (CM1; polyclonal antibody, Novocastra, Newcastle, UK) immunopositive cells were scattered in both the primary and metastatic sites (Fig. 6). Negative controls were prepared by replacing the primary antibody with non-immunized bovine serum. The specificity of the PTHrP antibody was confirmed by a preabsorption test with the corresponding PTHrP. The presence of cytoplasmic RNA was confirmed by methyl green pyronine staining of prepared sections (Muto Pure Chemicals, Tokyo, Japan).

Discussion

PTHrP play a physiological role in: (1) the regulation of cellular proliferation and differentiation, (2) fetal development, (3) the relaxation of vascular and non-vascular smooth muscle. The expression of the PTHrP gene in normal tissues is tightly regulated by a number of factors, involving both transcriptional and post-transcriptional mechanisms. In neoplastic proliferation, PTHrP is involved in the regulation of local tumor growth. Burton et al.[13] have shown that PTHrP may serve as an autocrine growth factor in human renal cell carcinoma. In the present case, it appears that both the primary and the metastatic tumor cells may have been

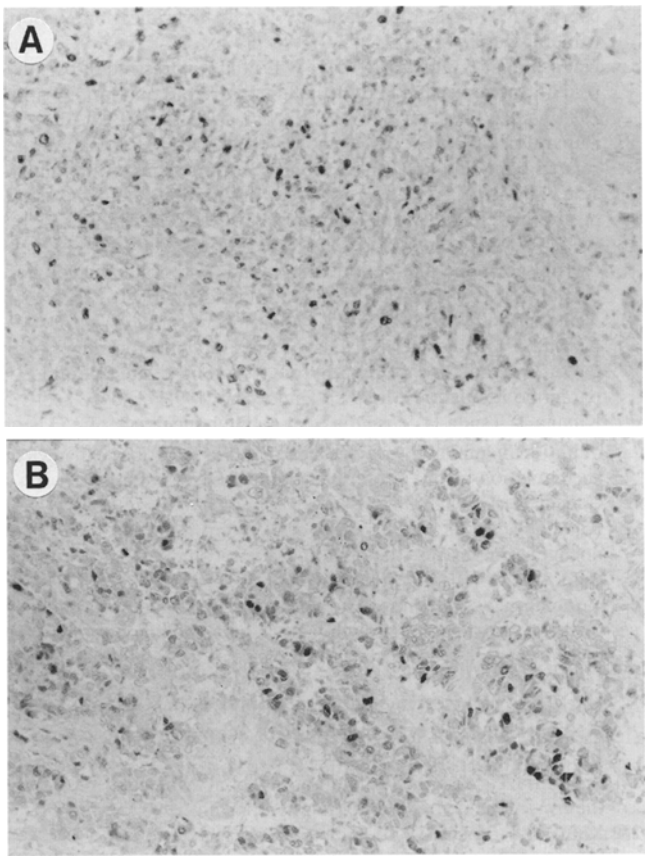

Fig. 6A,B. p53-Positive tumor cells were scattered in both **A** the primary gastric tumor and **B** the metastatic tumor cells of retroperitoneum. ABC method, ×50

stimulated to proliferate by PTHrP through the PTH/PTHrP receptor in a humoral or an autocrine/paracrine fashion. Hypercalcemia would have been a complication secondary to metastasis.

Varying sizes of PTHrP mRNA are synthesized depending on alternating splicing, and three types of mature peptides (N-terminal, midportion, and C-terminal) are produced from the propeptide by a prohormone convertase.[14] Immunodetection by an antibody encoding the N-terminal residue was very low in gastric cancer not associated with hypercalcemia.[10] Recently, Abdeen et al.[12] demonstrated that 93% of gastric adenocarcinomas (in 13 of 14 patients) showed immunopositivity for a monoclonal antibody encoding the C-terminal residue of PTHrP, irrespective of the presence of hypercalcemia. In the present case, PTHrP expression was confirmed, by immunodetection of an antibody encoding the midportion of PTHrP, only in the metastatic sites, but not in the primary gastric tumor. This discrepancy in PTHrP expression between primary and metastatic sites cannot be explained clearly. Several reports suggest the significance of PTHrP as a factor in tumor progression. Increased expression of PTHrP has been reported in skeletal metastasis of human breast cancer[15] and in advanced prostatic[8] and thyroid cancers.[16] Intense immunostaining for PTHrP was also documented in spinal metastases of growth hormone-producing pituitary tumors and in the invasive components of pituitary tumor,[7] and a newly established metastatic rat pituitary tumor possessed a malignant phenotype which had an enhanced level of PTHrP compared with the original non-metastatic GH3 tumor.[6] Luparello et al.[17] indicated that 8701-BC cells, derived from PTHrP-producing breast carcinoma, produced extracellular proteolytic enzymes, and they hypothesized that PTHrP could intervene in the local control of the invasive process in breast carcinoma.

Possible explanations of the discrepancy in PTHrP expression between the primary and metastatic sites in the case presented here would seem to be: (1) Only metastatic clonal cells acquired abnormal gene expression of PTHrP; (2) positive transcriptional and post-transcriptional regulation was triggered by cytokines in the metastatic tissues. For instance, transforming growth factor (TGF)-β and epithelial growth factor are known to stimulate PTHrP gene expression;[18,19] (3) the producing peptides were different in the primary and metastatic sites, and it was the midportion of PTHrP that was detected immunohistologically. The first and second explanations seem much more likely, than the third, because N-terminal and C-terminal portions were detected, by in situ hybridization and by serological analysis, respectively.

Although many oncogenes may be involved in tumorigenesis, such as *p53*,[20] *k-ras*,[21] and *TGF*-α,[22] only a limited number of critical molecular markers of advanced malignant transformation (including metastatic potential) in gastric cancer have been clarified, such as nm23,[23] hst-1,[24] $ERBB_2$,[25] fibroblast growth factor receptor,[26] and c-met.[27] In the present case, the expression of the anti-metastatic genes *nm23* and *p53* in primary and metastatic tumor cells did not differ.

In conclusion, this case suggests that the coexpression of PTHrP and PTH/PTHrP receptor plays an important role in tumor progression of gastric cancer, apart from acting as a causative factor of humoral hypercalcemia.

Acknowledgments. This study was supported in part by the Japanese Ministry of Education (Grant No. 07807026).

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