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EXPRESSION OF PARATHYROID HORMONE-RELATED PROTEIN mRNA IN TUMORS OBTAINED FROM PATIENTS WITH HUMORAL HYPERCALCEMIA OF MALIGNANCY

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Human parathyroid hormone-related protein (PTHrP) mRNA was detected in all fresh cancer tissues consecutively obtained from 6 patients with humoral hypercalcemia of malignancy (HHM). The primary sites of the cancers in these cases were distributed among different organs including the lung, esophagus, kidney and ovary. PTHrP mRNA was undetectable in all 10 fresh cancer tissues obtained from normocalcemic patients. These results suggest PTHrP as a major cause of HHM.

Key words: Parathyroid hormone-related protein — Parathyroid hormone — Hypercalcemia — Paraneoplastic syndrome — Ectopic hormone production

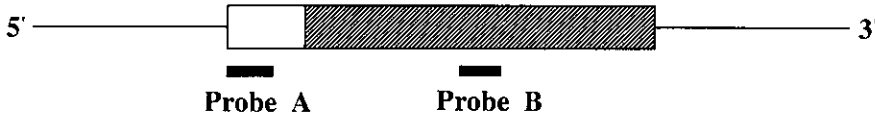
Hypercalcemia is one of the most frequent paraneoplastic syndromes in cancer patients; Yendt examined 438 consecutive patients with various malignancies and found hypercalcemia in 40 cases (9.1%).[1] Among several causes of hypercalcemia, the production of hypercalcemic factors by solid cancers, which is called humoral hypercalcemia of malignancy (HHM),[2] plays an important role in developing this electrolyte imbalance in cancer patients. Although the actual factor(s) responsible for HHM are not yet definitely identified, most investigators now believe that the majority of HHMs are caused by a humoral factor with parathyroid hormone (PTH)-like activity.[2–5] Recently a protein with PTH-like activity, termed PTH-related protein (PTHrP), was isolated from the culture medium of a squamous cell lung carcinoma cell line established from a patient with hypercalcemia.[6] A cDNA encoding this protein was identified, and its nucleotide sequence was determined.[7] Two other study groups purified identical proteins.[8–10] These findings suggest that PTHrP is a possible factor responsible for HHM. In the present study, we examined fresh cancer tissues consecutively obtained from patients with and without HHM for expression of PTHrP mRNA.

A total of 16 tumor tissues obtained at surgery were examined. Six of them were from hypercalcemic cancer patients. These were a squamous cell carcinoma of the lung, 2 squamous cell carcinomas of the esophagus, 2 renal adenocarcinomas and an ovarian cystadenocarcinoma. The clinical data (summarized in Table I) indicate that all these patients could be diagnosed as having HHM. Plasma PTH level was undetectable in 4 patients examined, as expected in cases with HHM. Furthermore, bone metastasis was not detected by X-ray examination and/or bone scintigraphy in any of the patients. As controls, 5 tumor tissues with histological types similar to those of patients with hypercalcemia were obtained at surgery from normocalcemic cancer patients. These were a squamous cell carcinoma of the lung, 2 squamous cell carcinomas of the esophagus and 2 renal adenocarcinomas. Five hepatocellular carcinoma tissues obtained at surgery from patients without hypercalcemia were also examined. Immediately after removal, the tissues were stored at $-80°$. Total cellular RNA was extracted by the method reported previously.[11] It was enriched in poly(A)$^+$ RNA by one cycle of oligo(dT)-cellulose affinity chromatography[12] prior to Northern

Table I. Clinical Data from Patients with Humoral Hypercalcemia of Malignancy

Patient no.	Age (yr)	Sex	Site of malignancy	Serum calcium level (mg/dl)	Plasma PTH level (ng/ml)
1	60	male	lung	14.3[a]	<0.2[b]
2	61	male	esophagus	12.0	<0.2
3	78	male	esophagus	16.2	<0.2
4	62	male	kidney	17.2	NT[c]
5	55	male	kidney	13.8	NT
6	44	female	ovary	16.7	<0.2

a) Normal range, 8.5–10.5 mg/dl.
b) Normal range, less than 1.3 ng/ml.
c) Not tested.



Probe A : 3' TAC GTC GCC TCT GAC CAA GTC GTC ACC TCG

CAG CGC CAC AAG GAC GAC TCG ATG CGC CAC 5'

Probe B : 3' CTA CTA CTC CCG TCT ATG GAT TGA GTC

CTT TGA TTG TTC CAC CTC TGC ATG 5'

Fig. 1. Structure of PTHrP mRNA and synthetic probes. The coding region and the untranslated region of the mRNA are shown by the box and by the single line, respectively. The hatched section of the box indicates the mature protein. The black rectangles below the mRNA structure show the regions where the 2 oligodeoxyribonucleotide probes can hybridize. Probe A (60 bases) corresponds to the amino-terminal 20 amino acids of the PTHrP precursor. Probe B (51 bases) corresponds to 17 amino acids of PTHrP(62–78).

blot hybridization. Synthetic oligodeoxyribonucleotides were designed as shown in Fig. 1, synthesized by the phosphoramidite method[13, 14] and purified by polyacrylamide gel electrophoresis.[15] The regions of PTHrP mRNA where the 2 oligodeoxyribonucleotide probes can hybridize have no significant homology with human PTH mRNA.[16] These probes were labeled with [γ-^{32}P]ATP as reported previously[11] and used as hybridization probes to identify PTHrP mRNA. Furthermore, a 39-base oligodeoxyribonucleotide probe complementary to human β-actin mRNA was used for determining the amount and the quality of mRNA used for the electrophoresis.[11] The specific activities of these labeled probes were about 4×10^6 cpm/pmol.

Northern blot hybridization was performed by the method reported previously.[11] When hybridized to probe A, 2 major bands with molecular sizes of 2.3 and 1.7 kb were detected in all lanes of tumor tissues obtained from patients with HHM. Furthermore, minor bands with larger molecular sizes were detected in most of these cases with HHM. In contrast, in all tumor tissues obtained from normocalcemic patients, no hybridizable band was detected (Fig. 2A). The bands were also

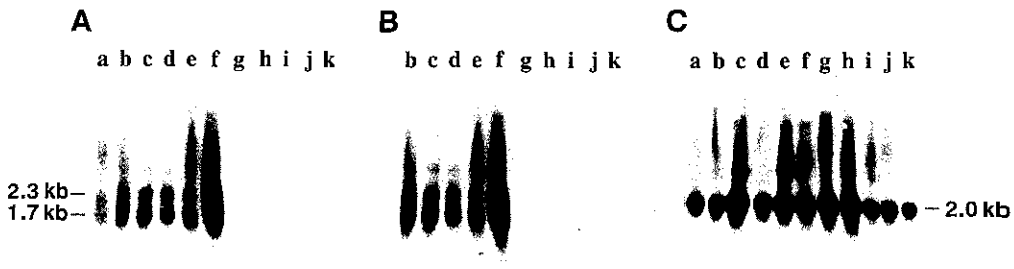


Fig. 2. Northern blot analyses for PTHrP and β-actin mRNA. Poly(A)$^+$ RNA (5 μg) prepared from tumor tissues obtained from hypercalcemic (lanes a, b, c, d, e, f) and normocalcemic (lanes g, h, i, j, k) patients were studied. The analysis was performed on the same nitrocellulose filter, except for one case (lane a) which was analyzed in a different experiment with only probe A and the probe for β-actin mRNA. The tumors were squamous cell carcinomas of the lung (lanes a, g), squamous cell carcinomas of the esophagus (lanes b, c, h, i), renal adenocarcinomas (lanes d, e, j, k) and an ovarian cystadenocarcinoma (lane f). (A) Results with probe A. (B) Results with probe B. (C) Results with the probe for β-actin mRNA.

undetectable in hepatocellular carcinoma tissues obtained from patients without hypercalcemia (data not shown). On hybridization to probe B, the same results were obtained (Fig. 2B). The probe for β-actin mRNA revealed a corresponding band in every tissue examined (Fig. 2C), indicating that poly(A)$^+$RNA prepared from these samples was of high enough quality, and that the amounts used for the electrophoresis were not very different among these tissues. It is reasonable to assume that the RNA detected in the present study was mRNA coding PTHrP, since the 2 different probes for PTHrP mRNA gave the same results in the Northern blot analyses, and the molecular sizes of mRNA detected corresponded to that of PTHrP mRNA.[7, 10] Further studies will be required to clarify the mechanisms of the expression of different sizes of PTHrP mRNA.

Several biological activities of PTHrP suggest that this protein is likely to be a candidate for the factor that causes HHM: the action and the structure of this protein bear striking resemblances to those of PTH[6–10, 17, 18]; this protein has bone-resorbing activity[6, 8]; administration of the amino-terminal fragment of PTHrP produces hypercalcemia in rats.[17] However, these findings do not necessarily mean that PTHrP is responsible for the syndrome of HHM in cancer patients. In the present study, we have detected the expression of PTHrP mRNA in all 6 tumor tissues consecutively obtained from patients with HHM, but not in 10 tumor tissues of normocalcemic patients. The fact that there is a very close relationship between expression of PTHrP mRNA and the development of HHM indicates that PTHrP is a major cause of hypercalcemia in these patients. Furthermore, it is worth noting that in this study PTHrP mRNA was detected in cancer tissues that originated from different organs including the lung, esophagus, kidney and ovary. As these cancers are representative ones frequently observed in patients with HHM,[3–5] it is likely that PTHrP plays a major role in the pathogenesis of HHM not just in a small proportion, but in most cases.

Recently, several factors with bone-resorbing activity have been found to be produced by cancer cells, and some of these factors, including transforming growth factors and interleukin-1α, were suggested to be the causative agents of HHM in some cases.[3, 19, 20] Since these factors do not possess PTH-like activity, a substantial contribution of these factors to the pathogenesis of HHM seems to be unlikely. In order to confirm directly that PTHrP is the actual causative agent of HHM, plasmas as well as tumors obtained from patients with HHM should be examined to see whether they contain enough PTHrP to develop hypercalcemia or not.

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