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PRODUCTION OF PARATHYROID HORMONE-RELATED PROTEIN IN ADULT T-CELL LEUKEMIA CELLS

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Human parathyroid hormone-related protein (PTHrP) mRNA was detected in peripheral leukemic cells obtained from adult T-cell leukemia (ATL) patients as well as in cultured human T-cell leukemia virus type I (HTLV-I)-infected T-cell lines. In contrast, PTHrP mRNA was not detected in other types of leukemic cells. Using radioimmunoassay, immunoreactive PTHrP was also detected in the spent media of HTLV-I-infected T-cell lines. These results suggest that PTHrP plays an important role in developing the hypercalcemia frequently observed in ATL patients.

Key words: Parathyroid hormone-related protein — Hypercalcemia — Adult T-cell leukemia — Human T-cell leukemia virus — Paraneoplastic syndrome

Adult T-cell leukemia (ATL) is a clinical entity characterized by the malignant growth of T4 antigen-positive peripheral T-lymphocytes. Extensive clinical studies have revealed that more than half of acute-type ATL patients developed hypercalcemia; this complication was rarely observed in patients with other types of leukemias.[1,2] Severe proliferation of osteoclasts was observed in pathological studies of bone tissues obtained from hypercalcemic ATL patients,[3] suggesting that a factor, or factors, with the ability to activate osteoclasts induced this phenomenon.[4,5] Although some reports have suggested that interleukin (IL)-1α or β is the possible causative agent of this abnormality,[5,6] the actual factor responsible for this electrolyte imbalance in ATL patients is not yet established. We report here evidence indicating that peripheral leukemic cells obtained from ATL patients as well as human T-cell leukemia virus type I (HTLV-I)-infected T-cell lines produce parathyroid hormone-related protein (PTHrP). This protein is a newly-discovered factor with calcium-elevating activity,[7] and is now considered to be the major causative agent of humoral hypercalcemia of malignancy (HHM),[8] the morbidity defined as the solid tumor-associated hypercalcemia induced by the production of hypercalcemic factor(s) by the tumor cells.

Six cultured cell lines associated with leukemia and 4 specimens of fresh leukemic cells obtained from patients were examined by Northern blot hybridization. Cultured cell lines were 3 HTLV-I-infected T-cell lines (MT-2, HUT-102 and MT-1),[9-11] one acute myelocytic leukemia (AML) cell line (ML-2),[12] one acute promyelocytic leukemia cell line (HL-60)[13] and one chronic myelocytic leukemia (CML) cell line (KOPM-28).[14] Three peripheral blood specimens obtained from 2 ATL and one CML patients and bone marrow cells obtained from one AML patient were also examined. One ATL patient was hypercalcemic (19.4 mg/dl) with a white-cell count of 205,000/mm^3 (abnormal lymphocytes, 67%); the other was normocalcemic (8.5 mg/dl) with a white-cell count of 97,000/mm^3 (abnormal lymphocytes, 85%). The AML patient was normocalcemic and more

than 90% of bone marrow cells in this patient were myeloblastic tumor cells. The CML patient in chronic state was normocalcemic, and the peripheral white-cell count was 367,000/mm^3 with 43% immature myeloid leukemic cells. The leukemic cells in peripheral blood of the 2 ATL patients and those in bone marrow of the AML patient were separated by Ficoll-Hypaque density gradient centrifugation.[15] The peripheral leukemic cells of the CML patient were isolated by the method reported previously.[16]

Poly(A)$^+$ RNA extraction, gel electrophoresis and Northern blot hybridization were performed by the method reported previously.[8] For detecting PTHrP mRNA, a previously described synthetic DNA probe, corresponding to 17 amino acids of PTHrP(62-78), was used.[8] The results of Northern blot hybridization for PTHrP mRNA are illustrated in Fig. 1. Among the 3 HTLV-I-infected T-cell lines, PTHrP mRNAs, composed of 2 major bands with molecular sizes of 2.3 and 1.7 kb, were detected in 2 cell lines. These bands were very similar to those detected in the solid tumor tissues from patients presenting with HHM as shown in the previous studies.[8] These bands were not detected in 3 leukemic cell lines other than ATL. In 2 specimens of peripheral leukemic cells obtained from ATL patients, both contained hybridizable bands. The intensity of the bands was stronger in the specimen obtained from the patient presenting with hypercalcemia. No band corresponding to PTHrP mRNA was observed in 2 specimens extracted from fresh tumor cells obtained from patients with AML and CML.

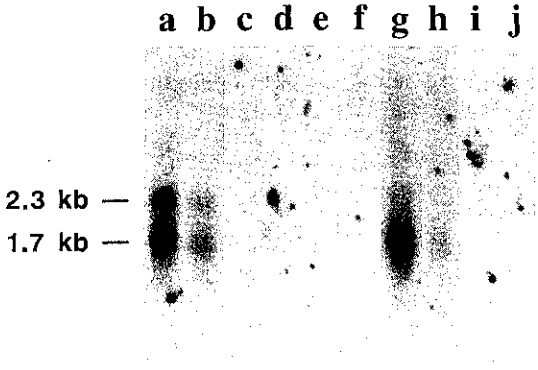

Fig. 1. Northern blot analysis for PTHrP mRNA. Poly(A)$^+$ RNAs (5 μg per lane) were prepared from HTLV-I-infected T-cell lines (a, MT-2; b, HUT-102; c, MT-1), leukemic cell lines other than ATL (d, ML-2; e, HL-60; f, KOPM-28), fresh leukemic cells obtained from ATL patients (g, hypercalcemic; h, normocalcemic) and fresh leukemic cells obtained from patients with leukemia other than ATL (i, AML; j, CML).

The production and the release of PTHrP by HTLV-I-infected T-cell lines were examined by a radioimmunoassay (RIA) in the spent media. The PTHrP RIA was performed by using antiserum against human PTHrP(1-34)amide which was synthesized by a solid-phase peptide synthesizer. Synthetic PTHrP (1-34) was purchased from Peninsula Laboratories (Belmont, Calif.) as a radioiodination and assay standard. Immunoreactive PTHrP was isolated from 10 ml of the spent media by immunoaffinity column chromatography which employed the same antiserum used for the RIA.[17] When 0.02 and 1.0 pmol of PTHrP(1-34) were extracted, the recovery rates (mean$\pm$SD) were 85.0$\pm$10.0 and 73.0 $\pm$2.3%, respectively. The minimum concentration of immunoreactive PTHrP detected in the spent media was 0.93p*M*. As positive controls, 3 cell lines established from solid tumor tissues obtained from patients presenting with HHM were also assessed. These were cell lines of pancreatic carcinoma (FA), melanoma (SEKI)[18] and esophageal carcinoma (KN-13); nude mice bearing these tumor cells developed hypercalcemia, and Northern

Table I. Concentrations of Immunoreactive PTHrP in the Spent Media of 6 Cultured Tumor Cell Lines

Cell lines	Immunoreactive PTHrP (p*M*)
HTLV-I-infected T-cell lines	
MT-2	93.00
HUT-102	2.50
MT-1	$<$0.93
HHM-associated cell lines	
KN-13 (esophageal ca.)[a]	570.00
SEKI (melanoma)	37.00
FA (pancreatic ca.)	25.00

a) ca., carcinoma.

blot analysis revealed that these tumor tissues expressed appreciable amounts of PTHrP mRNA (data not shown). As shown in Table I, immunoreactive PTHrP was detected in the spent media of 2 HTLV-I-infected T-cell lines, in which the expression of PTHrP mRNA was observed. Large amounts of immunoreactive PTHrP were detected in the spent media of 3 HHM-associated tumor cell lines. The dose-response curves of immunoreactive PTHrP prepared from these spent media were parallel to that of synthetic PTHrP(1-34), indicating that the active materials present in the spent media had a structure immunologically indistinguishable from PTHrP(1-34). It is reasonable to speculate that immunoreactive PTHrP present in the spent media of HTLV-I-infected T-cell lines is biologically active, for the following reasons; the RIA developed in this study recognizes the biologically active portion of PTHrP, and we have data indicating that the amount of PTHrP-like biological activity present in the spent media of HHM-associated tumor cell lines is well correlated with that of immunoreactivity detected by the present RIA (data not shown).

A recent report by Fukumoto *et al.* indicated that ATL patients presenting with hypercalcemia exhibited high nephrogenous cAMP, an indicator of parathyroid hormone (PTH)-like activity *in vivo*,[19] suggesting that undefined factor(s) with PTH-like activity may play an important role in developing hypercalcemia in these patients. In the case of HHM, PTHrP is now regarded as the causative factor responsible for this morbidity. The present study reveals that fresh leukemic cells obtained from ATL patients and HTLV-I-infected T-cell lines produced PTHrP and that the expression of PTHrP mRNA was limited to the fresh leukemic cells of ATL patients and HTLV-I-infected T-cell lines, as far as examined. Based on these findings, it is reasonable to assume that PTHrP could be the major factor responsible for hypercalcemia in ATL patients. At present, the actual mechanism of PTHrP production in ATL-related tumor cells is not known. It seems that infection of HTLV-I alone does not explain this phenomenon, because the ability to produce PTHrP is quite different in 3 HTLV-I-infected T-cell lines examined. These data are consistent with the clinical observations that hypercalcemia does not develop in all ATL patients. To clarify the actual mechanism of PTHrP production in ATL cells, it may be valuable to know whether PTHrP production is associated with T-cell malignancies other than ATL, as well as normal T-cells.

As far as other factors with bone-resorbing activity are concerned, IL-1α and β have been reported to be produced frequently by fresh leukemic cells of ATL patients and HTLV-I-infected T-cell lines.[5, 6] However, the production of IL-1s is not limited to leukemic cells of ATL patients.[20] Since IL-1α and β possess potent bone-resorbing activity, it is likely that IL-1s produced by ATL cells could aggravate hypercalcemia in ATL patients. Further studies are required to elucidate the synergistic effects of these hypercalcemic factors produced by ATL cells.

Addendum: Before the submission of this manuscript, T. Motokura *et al.*[21] reported the expression of PTHrP mRNA in one HTLV-I-infected T-cell line, MT-2. However, no information on PTHrP immunoreactivity or clinical materials was available.

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