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HYPERCALCEMIA IN CANCER

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Summary—Hypercalcemia may occur as a complication of haematological malignancies, in association with solid tumors with bone metastases, and with solid tumors in the absence of bone metastases. The latter syndrome, known as the humoral hypercalcemia of malignancy (HHM) shares many features with primary hyperparathyroidism. A parathyroid hormone-related protein (PTHrP) has been identified, isolated and cloned, which is most likely responsible for the calcium disturbances in HHM, PTHrP is a previously unrecognized hormone which has limited amino-terminal sequence homology with PTH and is the product of a separate gene. Tissue localization studies have identified PTHrP in squamous cell carcinomata, renal cortical carcinomata, in a proportion of breast cancers and in adult T-cell leukemia/lymphoma. In normal tissues, PTHrP has been immunohistochemically localized in keratinocytes, placenta and fetal parathyroid glands. In addition to its role in mediating hypercalcemia in cancer, PTHrP is likely to have an important endocrine role in the fetus, and perhaps a paracrine function in several organs.

INTRODUCTION

Hypercalcemia is a common complication of several different types of cancer, contributing to increased morbidity and mortality. Indeed malignancy is the most frequent cause of hypercalcemia in a general hospital inpatient population, whereas primary hyperparathyroidism is a more common cause of elevated blood calcium in the community at large [1].

The symptoms of hypercalcemia can be very disturbing, especially the profound nausea and anorexia which occur, the fluid loss which can lead to serious dehydration, and the central nervous system effects which can terminate in coma. Physicians caring for patients with cancer must constantly be aware of hypercalcemia as a possible complication. However, hypercalcemia should not be regarded as a terminal event in cancer, since it is often preventable and treatable in patients whose tumor and general condition can otherwise be managed satisfactorily [1].

HYPERCALCEMIA IN CANCER

Three main types of malignancy-associated hypercalcemia are recognized [1, 2]. The first of these is the elevated blood calcium often seen in patients with lytic bone metastases, usually from carcinoma of the breast, and also from renal cortical, lung and other tumors. The second type occurs frequently in certain hematological malignancies, particularly multiple myeloma, and in some lymphomas and leukemias. The third is the syndrome of hypercalcemia in patients without any or with minimal bony metastases, in whom the tumor produces a factor which acts generally upon the skeleton to promote bone resorption and upon the kidney to restrict calcium excretion. This latter syndrome has become known as humoral hypercalcemia of malignancy (HHM), and is frequently seen in patients with squamous cell carcinoma of the lung, other epithelial cancers, renal cortical carcinoma and some miscellaneous tumors [1, 2].

HUMORAL PRODUCTS OF CANCERS

In the 1920s it was recognized that certain cancers could be associated with high plasma calcium without evidence of bone metastases. Albright [3] suggested in 1941 that nonmetastic hypercalcemia in cancer could be due to "ectopic" production by the cancer of PTH because of the biochemical similarity to primary hyperparathyroidism [3]. This idea became established in the literature and persisted for many years [4]. When the first radioimmunoassays for PTH were established, the results

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seemed to support this view since detectable levels of PTH were found in patient's plasma and PTH-like immunoreactivity was detected in tumor extracts from several cancers from hypercalcemic patients. With the improved assays of the 1970s, however, evidence appeared that the tumor immunoreactivity differed from that of PTH (reviewed in [2, 5]). Indeed, several well-characterized antisera failed to detect any PTH in plasma or tumors from the cancer patients. Therefore, it seemed that the tumor factor contributing to this syndrome might be structurally similar to PTH but nevertheless be a separate protein.

Three landmark clinical studies of the early 1980s pointed out that the similarity between HHM and primary hyperparathyroidism was even greater than previously recognized, in that the cancer patients also had increased urinary cyclic AMP levels [6–8] despite the fact that their PTH levels were low or undetectable. Thus it had to be concluded that something was produced by these cancers which was not PTH itself, but which very closely resembled PTH in its actions. A search to identify the PTH-like agent responsible for HHM began, concluding in 1987 with the purification, cloning, sequencing and expression of a previously unrecognized PTH-related protein (PTHrP), [9, 10], and determination of the structure of cDNA clones which represent the alternative mRNA transcripts of the PTHrP gene [11, 12].

CHARACTERIZATION OF PTHrP

Purification of PTHrP was achieved from conditioned medium produced by a lung cancer cell line originally derived from a patient with squamous cell carcinoma of the lung, associated with hypercalcemia [9]. The properties of this protein are similar to the activities described for tumor extracts from patients and animals with HHM [13, 14] and in the culture medium from one such tumor cell line [15]. PTHrP resembles PTH in many of its biological actions, in that it stimulates cAMP production only in PTH target tissues and this action is prevented by antagonists of PTH. PTHrP is immunologically distinct from PTH since antisera against PTH which completely inhibit the biological effects of PTH have no effect on the activity of PTHrP [16].

The limited homology at the amino-terminal region of the mature protein (8 of the first 13 identical) between PTHrP and PTH seemed sufficient to account for the similar actions of PTHrP and PTH, and indeed the amino-terminal region of PTH interacts with the PTH receptor [17, 18]. In support of this idea, a synthetic PTHrP peptide consisting of the amino-terminal 34 residues was found to be more potent than the equivalent bovine or human PTH peptides in simulating cAMP formation in PTH-responsive osteogenic sarcoma cells [17]. Both purified and recombinant PTHrP are also more potent than either bovine or human PTH peptides in the same assay [16]. These studies have also shown that, like PTH, PTHrP peptides of <30 residues from the amino-terminus have substantially less biological activity, indicating the conformational importance of the amino-terminal region [17].

Although PTH and PTHrP have similar biological activities, they differ substantially at the molecular level. For example, cDNAs encoding PTHrP have little DNA sequence homology with PTH, a surprising result in view of their amino-terminal amino acid homology. In addition, the cDNA cloning of PTHrP revealed the existence of two distinct types of 5′-untranslated sequences [10]. This, along with the detection of multiple PTHrP mRNA species, suggested the potential involvement of an alternate splicing mechanism in PTHrP gene expression which is not involved in the expression of the PTH gene.

HUMAN PTHrP GENE

PTHrP cDNA clones have been isolated from libraries derived from a human lung cancer cell line [10] and two human renal carcinoma cell lines [11, 12]. They have been shown to contain essentially the same PTHrP coding sequence. However, the cDNAs differed in that they contained divergent carboxy-terminal coding regions and 5′- and 3′-untranslated regions. This indicated that alternatively spliced mRNAs produce multiple cDNA species encoding PTHrP.

The biochemical similarities between PTHrP and PTH suggested that the structure of the gene encoding PTHrP could well be similar to the gene encoding human PTH [11]. The position of the exons in the 5′ end of the PTHrP gene were determined by comparing the sequences of the alternate PTHrP cDNA clones with the intron–exon boundaries of the human PTH gene. Despite the similarity in exon structure—which is further evidence for the

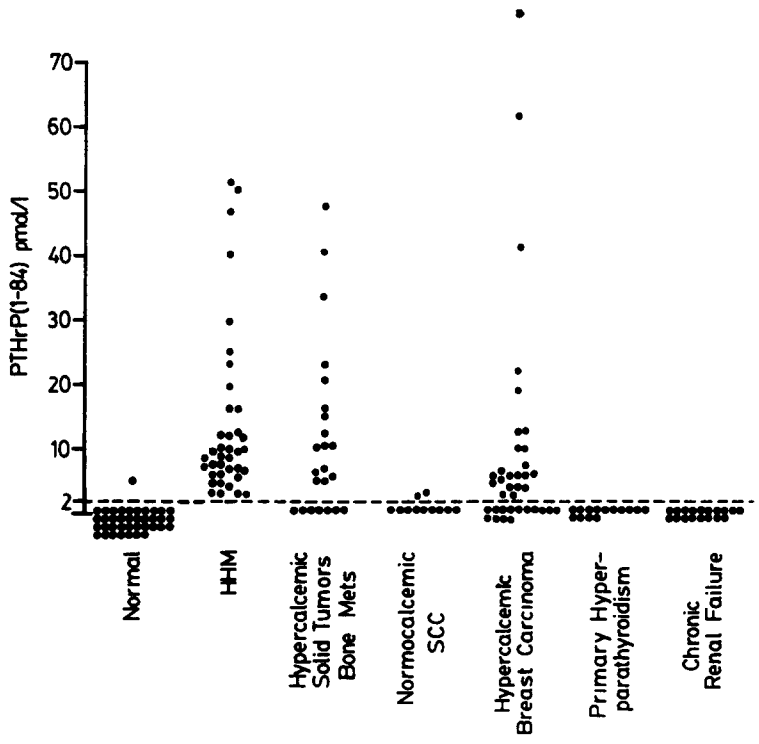

Fig. 1. Plasma levels of PTHrP in patients with various disease states (assay as in [22]).

relatedness of the two genes—the intron–exon arrangement of the PTHrP gene is clearly more complex than that of the PTH gene [18–21]. In addition to the variable 3′ exonic regions, production of multiple mRNA species by the action of alternate 5′ promoters is evident. Thus multiple promoter sites for PTHrP in conjunction with 3′ splicing events account for the multiple mRNA species in different tissues and cancers [21]. The use of these alternate promoters indicates complex regulation, and it remains to be determined whether this takes place in a tissue-specific manner.

HHM ROLE OF PTHrP

There seems little doubt that PTHrP is the major, if not the sole mediator of hypercalcemia in patients with the HHM syndrome. However it is still possible that in some patients, other bone-resorbing factors (e.g. interleukin-1, tumor necrosis factor, transforming growth factor alpha) could contribute to the development of hypercalcemia on a humoral basis. Although current radioimmunoassays are not sensitive enough to identify PTHrP convincingly in the plasma of normal subjects, elevated levels of PTHrP are detected in a high proportion of patients with hypercalcemia in cancer ([22], Fig. 1). Furthermore, antibodies to N-terminal regions of PTHrP can reduce the hypercalcemia and prevent the bone abnormalities in a nude mouse model of HHM [23]. There are however, some discrepancies between the features of HHM and hyperparathyroidism [1] which may relate either to interactions with other tumor factors which may be co-secreted with PTHrP, or possibly to actions mediated via regions of the PTHrP molecule beyond the first 34 amino-acids. For example we have noted altered renal handling of bicarbonate by the rat kidney perfused with PTHrP (1–141) as compared to PTHrP (1–34) [23].

PTHrP has been detected immunohistochemically and by Northern blotting in many tumors (commonly of squamous epithelial origin) which give rise to HHM [21, 24]. It has also been detected in parathyroid adenomata, breast tumors from normocalcemic patients [25], hypertrophic breast tissue and in a percentage of hypercalcemic patients with HTLV 1 lymphomas. In normal subjects PTHrP can be identified in high turnover epithelial tissues in skin and lactating breast (and breast milk) [24, 26]. It is also present in many fetal tissues including fetal epithelia, muscle, bone,

kidney, parathyroids and placenta [27], and its expression by embryonal carcinoma cells suggests a possible role in developmental processes. A role for PTHrP in placental calcium transport has been demonstrated, located beyond the first 34 amino-acids but within PTHrP (1–84) [28, 29]. Such an action may also have relevance to calcium transport into milk by breast epithelium, and thus also in the local control of the calcium environment critical for epithelial growth and differentiation. This hypothesis is supported by localization studies which indicate its presence in highest concentrations in areas of active growth in normal and cancerous epithelial cells and in normal skin which undergoes rapid turnover.

Whether PTHrP actually circulates as a functioning hormone in post-natal animals and man is unknown at present. It may be that only vanishingly small amounts of PTHrP reach the circulation in normal subject, and that it functions as a hormone only in the fetus and in those pathological states where excess production and secretion occur. If this is so, the value of PTHrP assay is a tumor marker is likely to be considerable.

We do not yet know the nature of circulating PTHrP in patients with cancer. The many potential cleavage sites in the molecule suggest that circulating forms will be quite heterogeneous. Different tumors could process the molecule at varying sites, a possibility consistent with experience during purification efforts in various laboratories.

It is known now that PTHrP is produced by all squamous cell cancers and a number of other tumors as well. Only a proportion of these patients become hypercalcemic, however, and the determining factors are probably the amount of PTHrP which gets into the circulation, and the capacity of the normal homeostatic control mechanisms to regulate the calcium levels. It should be predicted that in squamous cell carcinoma of the lung, for example, plasma PTHrP levels would be elevated above normal before any other biochemical effects are seen—certainly before hypercalcemia, and possibly before significant effects on urinary cyclic AMP, phosphorus or calcium. Thus measuring PTHrP would be valuable in the early detection of lung cancer and in treatment monitoring. This is especially important for squamous cell carcinoma of the lung, and also in other cancers including renal cortical carcinoma, certain groups of patients with breast cancer, a number of epithelial cancers, and in adult T-cell leukemia [30]. In view of the location of PTHrP in abnormal parathyroid diseases, both primary and secondary, the role of PTHrP in these conditions needs to be explored.

HYPERCALCAEMIA ASSOCIATED WITH BONE METASTASES

Despite many improvements in early cancer detection and more effective treatment, metastatic disease remains the leading cause of cancer-related deaths. Bone is the most common site of metastasis in breast cancer and 25% of early stage patients will develop this complication. This figure increases to 75% in patients with advanced disease [31]. Currently there is no single, accurate predictor to identify which patients will develop this complication. In the clinical follow-up of patients with breast cancer especially, bone scanning at regular intervals is important in the detection of bone metastases. Recognition of the symptoms of early hypercalcemia is of the utmost importance, since further progression can be prevented by appropriate measures, including increase fluid intake, and antitumor therapy.

Although for many years it was considered that the main mechanism of hypercalcemia in patients with breast cancer was the release of calcium from bone by osteolytic deposits [1], there is increasing evidence for a humoral contribution in these patients also. The extent of metastatic bone disease correlates poorly with both the occurrence and the degree of hypercalcemia in malignancy [32]. In 80–90% of cases of unselected solid tumor patients with hypercalcemia, irrespective of whether bony metastases are present, there is evidence of an underlying humoral mechanism. The putative humoral mediator predisposes to hypercalcemia both by stimulating generalized osteolysis and, in most cases, by impairing the renal excretion of the resultant increase in filtered calcium load. A reduced renal phosphate threshold and increased tubular calcium reabsorption were observed in hypercalcemic patients when compared with their normocalcemic counterparts, emphasising the importance of renal mechanisms in mediating the hypercalcemia.

In some studies patients with breast cancer have been identified with biochemical features of HHM [33, 34]. PTHrP has been purified from a breast cancer [35] and we have evidence by

immunohistology for the presence of PTHrP in 60% of an unselected series of breast cancers [36]. Furthermore, PTHrP is produced by the lactating breast and elevated plasma PTHrP levels have been found in a patient with lactational hypercalcemia [36]. All of these observations focus on the need for further study of the role of PTHrP in malignant and nonmalignant breast disease.

It is therefore of particular interest that 65% of patients with hypercalcemia associated with breast cancer and bone metastases were found to have elevated levels of PTHrP in plasma (Fig. 1, [22]). This might reflect a humoral component in the hypercalcemia of breast cancer. It is also possible, given the fact that PTHrP is produced commonly by breast cancers [36], that PTHrP produced by breast cancers might contribute to their ability to establish and grow in bone. Preliminary evidence in support of this comes from a study showing enrichment of PTHrP by immunohistology in metastatic breast cancer deposits in bone [31].

HEMATOLOGICAL MALIGNANCIES

Hematological malignancies are frequently associated with osteolytic bone destruction and with hypercalcemia. Lympho-proliferative disorders such as malignant lymphoma, and occasionally patients with chronic myeloid leukemia and acute lymphoblastic leukemia develop hypercalcemia. Hypercalcemia occurs in approximately one third of all patients with multiple myeloma [1], a disease resulting from uncontrolled proliferation of plasma cells derived from a single clone. Bone involvement in myeloma is characterized by extensive bone destruction accompanied by pain and susceptibility to fracture. Skeletal X-rays reveal abnormalities in 79% of patients. These consist of osteoporosis, lytic lesions and fractures with over half of the patients having a combination of all three.

The mechanism of bone resorption leading to hypercalcemia in myeloma is due to the secretion by myeloma cells of bone resorbing cytokines which stimulates osteoclasts. A number of cytokines previously described by the generic name "osteoclast-activating factor" have now been identified in activated leukocyte cultures: interleukin-1, tumor necrosis factor α (cachectin) and tumor necrosis factor β (lymphotoxin). It is not yet certain whether one or more of these cytokines are responsible for the stimulation of osteoclastic bone resorption in multiple myeloma. A recent study found that most, but not all, of the bone resorbing activity from the human myeloma cell line could be suppressed by neutralizing antibodies to lymphotoxin [37]. Although production of this bone-resorbing cytokine may be related to osteoclastic bone destruction and hypercalcemia in patients with myeloma, other cytokines may also be involved.

Hypercalcemia is uncommon in both non-Hodgkin's or Hodgkin's lymphoma. Although most often associated with bone involvement and related to direct tumor invasion of bone, it can also occur in the absence of lytic lesions in bone, consistent with a humoral mechanism. Several case reports have identified patients with both Hodgkin's and nonHodgkin's lymphoma and hypercalcemia with no clinical or radiographic evidence of cortical bone involvement who had elevated levels of 1,25(OH)2D [38, 39]. These patients also had suppressed immunoreactive PTH levels. Circumstantial evidence for lymphomatous tissue as the site for extra-renal synthesis of 1,25(OH)2D is provided by the observation that the institution of effective antitumor chemotherapy regimens resulted in a substantial decrease in the circulating levels of 1,25(OH)2D [40]. Similarly, surgical excision of a solitary splenic lymphoma resulted in resolution of the hypercalcemia in another reported case [41].

ADULT T-CELL LEUKEMIA/LYMPHOMA

Although hypercalcemia is an infrequent complication of lymphoma, a particular diagnostic sub group of patients with adult T-cell lymphoma has a very high incidence of hypercalcemia, varying from 26 to 100% in different reports. It is interesting to note that serum 1,25(OH)2D levels in this group of patients are uniformly suppressed [39, 42]. This disease has predominant geographic distribution in Japan and a small cluster in the West Indies. It is strongly associated with a retrovirus, human T-cell lymphotrophic virus type 1 (HTLV-1). Hypercalcemia is the most important prognostic determinant in this disease and also a frequent cause of death. In contrast to patients with B-cell lymphoma, hypercalcemia in these patients is associated with elevated nephrogenous cyclic adenosine monophosphate (NcAMP) levels, low-normal immunoreactive parathyroid hormone (iPTH) levels, and re-

duced serum 1,25(OH)2D concentrations [43], in the absence of lytic lesions in bone. These features are similar to those seen in the syndrome of HHM. Conditioned media from cultures of HTLV-1 infected cells, and of peripheral lymphocytes from a hypercalcemic patient with this disorder were found to contain PTH-like biological activity [43]. It is shown also that a PTH-like biological activity was synthesized and secreted by these cells, which had all the expected properties of PTHrP. These workers also demonstrated expression of PTHrP within HTLV-1-infected T-cells in culture [30]. They also reported PTH-like biological activity present in pleural and ascitic fluid from patients with ATLL and hypercalcemia [30]. These findings confirmed their hypothesis that PTHrP is produced by tumor cells in adult T-cell leukemia/lymphoma, and that this may be an important factor in the development of hypercalcemia in this disease.

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