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Advances in Brief

Localization of Parathyroid Hormone-related Protein in Breast Cancer Metastases: Increased Incidence in Bone Compared with Other Sites[1]

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Abstract

Parathyroid hormone-related protein (PTHrP) has recently been identified in 60% of a series of primary breast cancers. The detection of a bone-resorbing factor in tumors with a propensity to metastasize to bone prompted study of PTHrP in breast cancer metastasis. PTHrP was localized by immunohistology in 12 of 13 (92%) breast cancer metastases in bone and in 3 of 18 (17%) metastases in non-bone sites. The statistical difference was highly significant ($P < 0.0001$). Production of PTHrP as a bone-resorbing agent may contribute to the ability of breast cancers to grow as bone metastases.

Introduction

PTHrP[6] is recognized as the principal agent responsible for the increased bone resorption and reduced calcium excretion accompanying the humoral hypercalcemia of malignancy (1). In addition, it has been identified in normal human skin (2), as well as in a series of squamous cell cancers in normocalcemic subjects (2). We have demonstrated PTHrP immunohistochemically in 60% of primary tumors from 101 unselected patients with breast cancer (3). Seven of these patients have developed bone metastases in the early period of follow-up and all have had primary tumors that have stained positively for PTHrP. These observations and the frequency with which breast cancer metastasizes to bone (4) prompted the present investigation, in which immunohistochemistry has been used to determine the incidence of PTHrP staining in a retrospective series of breast cancer metastases in bone and non-bone sites. The metastatic process is a complex, nonstochastic sequence of essential steps, in which a metastatic cancer cell must interact with the microenvironment of the target organ in a manner that allows tumor growth if this process is to be completed. PTHrP is a powerful stimulator of bone resorption (5). This has led us to consider whether PTHrP produced by breast cancers might contribute to their ability to establish and grow in bone.

Materials and Methods

Paraffin blocks of breast cancer metastases were obtained from pathology archives for all biopsy-proved metastases. Those arising from breast primaries were positively identified and metastases to regional lymph nodes and local recurrences were excluded from study. Postmortem specimens were also excluded inasmuch as these have previously been found to be unsuitable for immunohistochemistry due to the effects of autolysis. The patient's records were used to confirm the findings and to provide relevant clinical data. A total of 42 biopsy-proved, distant breast cancer metastases were identified from 40 patients over the period 1986 to 1990 for which there were complete records. Of these, sufficient material was available for immunohistochemical study in 31 cases.

Tumor Samples. In the case of soft tissue metastases, the tumor tissue had been formalin fixed, routinely processed, and embedded in paraffin. Tissue samples taken from bone had been decalcified in citric (2.5%)/formic acid (8.75%) solution (6) following fixation before processing and paraffin embedding. Sections (5 μm thick) at either end of those cut serially were stained in hematoxylin and eosin and reviewed by a pathologist to ensure that representative tumor was present in the sections.

Immunohistochemistry. The primary antibody was a polyclonal antiserum raised against the amino-terminal portion of the PTHrP molecule by injecting synthetic PTHrP(1–34) into New Zealand White rabbits. The antiserum (255.5) was characterized as described previously (2, 3) and demonstrated no detectable cross-reactivity with parathyroid hormone. This antiserum compares favorably with other PTHrP antisera used previously in immunohistochemistry (2, 7). The peroxidase-antiperoxidase method of tissue antigen localization used was modified from that described by Danks *et al.* (2), based on the technique of Sternberger *et al.* (8).

Controls. To ensure specificity of staining for PTHrP, antibody and method controls were performed (2) including *(a)* deletion of alternate layers of the antibody sandwich (primary antiserum, secondary antiserum, and PAP complex) and *(b)* preabsorption of anti-PTHrP(1–34) antiserum overnight with 0.5 mg/ml synthetic PTHrP(1–34) and human PTH(1–34), respectively, prior to immunohistochemistry on skin and breast cancer specimens previously found to stain positively for PTHrP. To ascertain that the decalcification technique did not alter PTHrP staining, a fresh parathyroid adenoma was placed in decalcification solution, processed, and used as a positive control for immunostaining (7). All experiments included samples of human skin as a positive control (2) and the substitution of primary antibody with preimmune rabbit serum as a negative control.

Evaluation of Staining. The stained sections were reviewed by three independent observers and compared with controls and hematoxylin and eosin-stained slides. Tumor metastases were called positive if any of the tumor cells stained brown. Sections involving bone marrow were reviewed by a pathologist to ensure that marrow stem cells were not confused with tumor. There was concordance of opinion in all cases except one specimen from bone marrow which was equivocal and therefore judged to be negative.

Results

Of the 31 specimens obtained for study, 13 were from bone and 18 were from various non-bone sites (Table 1). The 13 bone metastases included 6 diagnostic trephines, reamings from 4

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[6] The abbreviation used is: PTHrP, parathyroid hormone-related protein.

Table 1 *Frequency with which PTHrP was detected by immunohistochemistry in breast cancer metastases from bone and non-bone sites*

Site of metastasis	Total no.	No. positive	No. negative
Bone	13	12	1
Extradural	5	0	5
Lung/pleura	4	2	2
i.p.	4	0	4
Skin	2	1	1
Liver	1	0	1
Brain	1	0	1
Pericardium	1	0	1
Total	32	15	17

fractures that were pinned surgically, and 3 intact specimens from bones removed at joint replacement or spinal decompression surgery. The tissue from non-bone sites was obtained in all instances either from diagnostic biopsy or at open operation for palliation of compression syndromes.

Fig. 1 shows an example of a positively stained metastatic deposit in bone whereas Fig. 2 displays a control section showing no staining. Positive staining was seen in 12 of 13 (92.3%) metastases in bone compared with 3 of 18 (16.7%) non-bone metastases ($P < 0.0001$, χ^2 test). Table 1 shows the results of PTHrP staining by site of metastasis for the 31 secondary tumors stained. The positive results among the non-bone metastases were seen in one of two s.c. metastases to distant skin sites and two of four metastases to lung or the pleural space. Biopsy-proved metastases to bone and non-bone sites from the same patient were not identified for comparison in this study.

Deletion of any layer of the antibody sandwich abolished positive staining. Preabsorption of primary antibody with PTHrP(1–34) at a concentration of 0.5 mg/ml almost entirely abolished immunostaining whereas preabsorption with parathyroid hormone under identical conditions did not diminish positive staining compared with controls. Immunohistochemistry of parathyroid adenoma treated in decalcifying solution showed

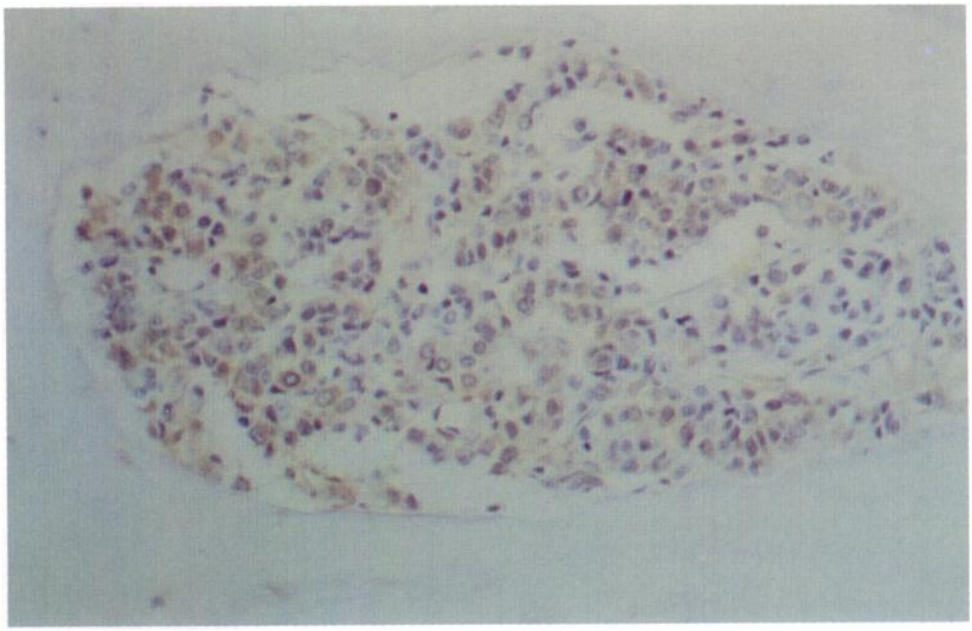

Fig. 1. Immunoperoxidase staining of a metastatic invasive ductal carcinoma of the breast deposit in bone with anti-PTHrP(1–34). The tumor cells show brown staining by peroxidase on diaminobenzidine while surrounding bone trabecula are counterstained blue with Harris' hematoxylin.

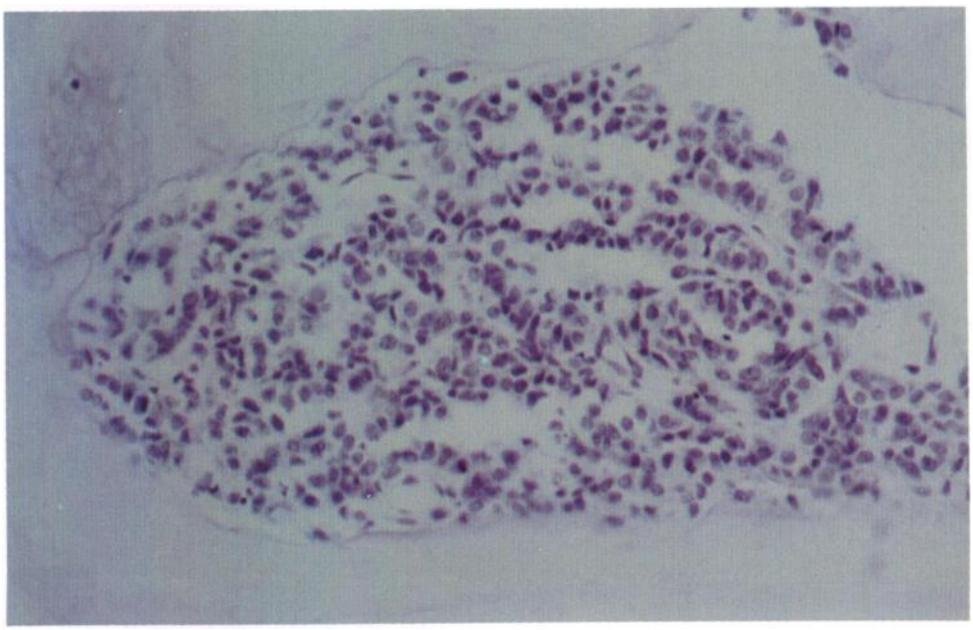

Fig. 2. Control section of the same tumor deposit in which preimmune rabbit serum has been substituted for anti-PTHrP(1–34). Tumor staining for PTHrP is not present.

no diminution of positive staining compared with that processed routinely.

Discussion

This retrospective study shows a highly significant difference in PTHrP localization in skeletal and nonskeletal metastases from breast cancers. This high incidence of PTHrP-positive tumor tissue in tumor deposits in bone raises the possibility that PTHrP-producing cells of the tumors are predisposed to growing in bone. Although the present study does not exclude the possibility that PTHrP staining was the result of some interaction of the tumor cells within the bone environment, this seems unlikely, especially inasmuch as it has already been shown that 60% of primary breast cancers are PTHrP positive by immunohistology (3).

In the present series we were unable to study suitable histological material from more than a few of the primary tumors from which the metastases originated, because the original surgery had been performed up to 21 years earlier in many other centers, where tissue handling was not uniform. The predictive value of PTHrP staining of primary breast cancers will require prospective study, such as that being undertaken in the reported series (3), in which the seven patients who to the present time have metastases in bone all had primary tumors which stained positively for PTHrP.

Despite many improvements in early cancer detection and more effective treatment, metastatic disease remains the leading cause of cancer-related deaths (9). Bone is the most common site of metastasis in breast cancer (10) and 25% of early stage patients will develop this complication. This figure increases to 75% in patients with advanced disease (11). Currently there is no single, accurate predictor to identify which patients will develop this complication (12). If PTHrP production by primary tumors provides a means of identifying those at risk for development of bone involvement, it would allow selection of those most likely to benefit from specific treatments aimed at preventing bone metastases, *e.g.*, bisphosphonates (13).

The process of tumor metastasis is not random but results from a series of complex steps (9). Both clinical and experimental data suggest that cancer cells from a given primary tumor require special properties in order to grow in distant organs (14). Growth in bone would require the ability to promote bone resorption, which is a property of PTHrP and also of prostaglandin E and transforming growth factor α, both of which are also produced by breast (15, 16) and other cancers.

The predilection for bone metastasis formation at sites plentiful in red marrow has been recognized for more than a century (17). Osteoclastic bone resorption may be important in at least the early phase of osteolytic metastasis (18). The high incidence of PTHrP staining within bone metastases may reflect a survival advantage conferred on metastatic tumor cells that are able to produce PTHrP and are better able to invade the bone matrix due to induction of osteoclast activity. It is also possible that the environment of the bone marrow may favor activation of the PTHrP gene, thereby enabling bone invasion by tumor deposits that might otherwise lie dormant or succumb to host defense mechanisms.

Other bone-resorbing tumor products (*e.g.*, transforming growth factors, cytokines) could operate in concert with PTHrP to facilitate skeletal metastasis and should be considered in any prospective study of the role of PTHrP. There is increasing evidence for a humoral mechanism of hypercalcemia in breast cancer (19), and the accruing data suggest that bone resorption contributing to hypercalcemia in breast cancer patients is disproportionate to the tumor burden in bone (20). This phenomenon may also be attributable to local PTHrP production by breast cancer metastases.

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