Parathyroid Hormone–Related Protein Localization in Breast Cancers Predict Improved Prognosis


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Abstract

In a prospective study of 526 consecutive patients with operable breast cancer, the significance of positive parathyroid hormone–related protein (PTHrP) staining by immunohistology has been evaluated for a median of 10-year follow-up. Improved survival was observed for the 79% of tumors which stained positively for PTHrP [estimated univariate hazard ratio, 0.43; 95% confidence interval (95% CI), 0.30-0.62; \( P < 0.001 \)]. Adjustments for N stage, progesterone receptor status, and log tumor size changed this estimate only slightly to 0.47 (95% CI, 0.63-0.69; \( P = 0.001 \)). Patients with PTHrP-positive primary tumors were less likely to develop bone metastases (hazard ratio, 0.63; 95% CI, 0.41-0.98; \( P = 0.04 \)). PTHrP status was associated with estrogen receptor (\( P = 0.01 \)), progesterone receptor (\( P = 0.03 \), and menopausal status (\( P = 0.006 \)) but was not significantly associated with tumor size, vascular invasion, tumor grade, or patient age. Of 19 patients requiring surgery for bone metastases, the primary cancers were PTHrP negative in seven, all but one of whom had PTHrP-positive bone metastases. All 12 patients with PTHrP-positive primary cancers also had positive bone metastases. We conclude that increased production of PTHrP by breast cancers confers on them a less invasive phenotype, an effect distinct from the bone resorption–stimulating action that favors bone metastasis. It is likely that the latter property is influenced by factors in the bone microenvironment. (Cancer Res 2006; 66(4): 2250-6)

Introduction

Bone is by far the most common site of breast cancer metastasis and causes considerable morbidity (1). The predilection of breast cancers to grow as metastases in bone has been recognized since it was described by Paget (2). Paget’s insights were based on an extensive autopsy study in women with breast cancer in which he noted the remarkable frequency of secondary deposits in bone, especially at the ends of the femora and in the skull. This led him to develop the “seed and soil” hypothesis of metastatic growth, with bone as the favorable soil for the “seed” of metastatic breast cancer cells. This hypothesis still encapsulates modern views of the metastatic process: to establish and grow in distant organs, tumor cells require specific properties that suit them to those organs (3).

Bone as a target for metastasis presents a particularly harsh environment for the establishment and proliferation of cancer cells. In addition to their general invasive properties, cancer cells are required to adhere to the bone surface and to promote the formation of active osteoclasts from precursors in the host bone marrow, thereby initiating resorption and allowing the tumor to establish and expand (4–6).

The recognition that parathyroid hormone–related protein (PTHrP) was responsible for the humorally mediated hypercalcemia in many patients with malignancy (humoral hypercalcemia of malignancy) provided new insights into the skeletal complications of cancer. The hypercalcemic effect of PTHrP is derived from its parathyroid hormone–like ability to promote bone resorption and restrict calcium excretion. Subsequently, PTHrP was found by immunohistochemistry to be expressed in about two thirds of primary breast cancers (7) and plasma levels were elevated in 70% of women with breast cancer and bone metastases who were hypercalcemic (8). The finding that PTHrP was detected in 85% of breast cancer metastases to bone but only in 16% of those to other sites led to the suggestion that PTHrP production might be important in conferring on breast cancer cells the special property they require to establish and grow in bone (i.e., the ability to promote bone resorption; ref. 9). Experimental studies using a mouse model of bone metastases supported a role for PTHrP in the establishment and growth of bone metastases (6, 10–12).

This prospective study was prompted by findings, noted above (9), which implicated PTHrP in the development of bone metastases. Early results of the study suggested that patients with primary tumors that possessed detectable PTHrP had improved survival compared with those with tumors that lacked detectable PTHrP (13). By continuing the study and recruiting new patients, we have been able to test if the previous findings were replicable, and if so, by combining the data, derive enhanced precision for measuring the improved survival of patients with PTHrP-positive primary breast cancers.

Materials and Methods

Patients. The patients were a consecutive series of 526 patients with stage I to III operable breast cancer presenting between December 1, 1989 and December 31, 1996. These consisted of the original 367 patients presenting between December 1, 1989 and December 31, 1994 who had been followed until December 31, 2000 (14). Additional data were now derived from 159 new patients presenting between January 1, 1995 and December 31, 1996, with follow-up until December 31, 2004, in addition to further follow-up of the original cohort until the same date. All patients presenting to the Breast Unit in the University of Melbourne Department of Surgery at St. Vincent’s Hospital were eligible for the study. We excluded 47 patients because their tumors were purely ductal carcinoma in situ or microinvasive or the patient had a past history of other primary malignancy apart from nonmelanoma skin cancer. In addition, patients

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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who received preoperative systemic therapy were excluded because there was not sufficient representative tissue available for analysis of PTHrP before the commencement of treatment. All patients were followed for a minimum of 8 years (to December 2004) and the median length of follow-up was 10 years. Patients were followed prospectively for the timing and site of metastases, as well as local and regional recurrences and overall survival. All treatment decisions were made at the discretion of the treating clinicians but the majority of patients were treated according to protocols in existence at the time of first presentation. Patients were reviewed at least twice yearly for the first 3 years and then annually. During follow-up reviews, routine scans and blood tests were not done in most cases. The diagnosis of metastasis for this study was based on pathologic confirmation, unequivocal results of investigations confirming metastasis, or, in cases where the development of metastasis could not be completely confirmed, on the results of serial investigations leading to a decision to treat. The study was approved by the Human Research Ethics Committee of St. Vincent’s Hospital, Melbourne, Australia.

**Prognostic factors.** Tumor size and lymph node status were obtained from the original pathology report but tumor grade, the presence of lymphatic and/or vascular invasion, tumor type, and estrogen receptor (ER) and progesterone receptor (PR) immunohistochemistry were all assessed by the panel. Tumors were classified according to the criteria of the American Joint Commission on Cancer (AJCC) and disease was staged according to the AJCC classification (16). Tumors were graded using the Elston-Ellis modification of the Scarff-Bloom-Richardson classification (17). ER and PR status were assessed by immunohistochemistry using a standard peroxidase-antiperoxidase method (14), with polyclonal rabbit antiserum raised to the human PTHrP peptide containing amino acids 1 to 14 [PTHrP(1-14)]. The antiserum specificity has been characterized (14, 15) and no cross-reactivity with parathyroid hormone was observed under any condition examined, including Western blotting. The following methods and antibody controls were used: alternating deletion of the antibody layers, preabsorption of anti-PTHrP(1-14) overnight with peptide at 4°C, application of PTHrP(1-14) at 0.5 mg/mL to the tissue sections 5 minutes before the addition of the antiserum, and replacement of the anti-PTHrP(1-14) with nonimmune rabbit serum. Each assay included a positive control (normal skin). Each tumor section was stained in duplicate, with two dilutions of antiserum, and was assessed by a panel of individuals who were unaware of the clinical details. Tumors were called positive for PTHrP when specific staining was observed in any cell that was unequivocally identified as a tumor cell. In addition, a random series of samples (8%) that had been reviewed previously by the panel were restained and evaluated as an internal control of assessment. There was 100% concordance with the original assessments. The assay was repeated if there was substantial background staining on the nonimmune control, if there was discordance between the two stained sections, or if there was a lack of agreement among panel members. In all positive tumors, a cytoplasmic pattern was found. In 20% of these tumors, some specific membrane staining was also noted.

**Statistical methods.** Frequency data were assessed by standard contingency table analysis, including Fisher’s exact test, for binary or categorical variables and by the Mann-Whitney test for continuous variables. The strength of association between binary variables was measured by the odds ratio, with exact 95% confidence intervals (95% CI) described by Mehta (18). Kaplan-Meier survival curves were created for survival analysis and differences in survival were assessed using a Cox Proportional Hazards model (19, 20). Nested models were compared using the likelihood ratio test. The proportional hazards assumption was checked using the Grambsch-Therneau test (21) and data were stratified when necessary to satisfy this assumption. All reported significance levels are nominal and two sided. Following convention, tests with \( P < 0.05 \) were considered to be statistically significant.

Time to death was analyzed by Cox proportional hazards regression and the data were divided into two parts. The initial data related to patients presenting on between December 1, 1989 and December 31, 1994, with follow-up until December 31, 2000. Observations were made on 367 patients for \(-1,896\) person-years, during which 73 women died of breast cancer-related causes. The subsequent data related to patients presenting between January 1, 1995 and December 31, 1996, as well as to additional follow-up of the 270 women from the initial cohort, left censored at December 31, 2000 (22). Follow-up in this subsequent group continued until December 31, 2004. Whereas some of the women observed in the initial period of the study were also observed in the subsequent period, there was no overlap in observation, so the two data sets can be considered independent. There were 429 women observed during the subsequent period, for a total of \(-1,957\) person-years, during which 55 breast cancer-related deaths were observed. Combining the data provided observations on 526 women for a total of 3,789 person-years with 128 breast cancer deaths.

For each set of data, a univariate analysis was used to estimate the hazard ratio for PTHrP status. PTHrP status was then included in a multivariate analysis along with age, number of positive lymph nodes, PR status, ER status, and log tumor size. Those factors judged not to contribute to the model were removed to leave a final model.

### Results

Table 1 summarizes the clinical and pathologic characteristics of the 526 patients who had a median age of 60 years (range, 27-93 years): 72% were postmenopausal, 10% perimenopausal, and 18% premenopausal; 183 (35%) presented with AJCC stage I breast cancer, 161 (31%) with stage IIa, 71 (14%) with stage IIb, 77 (15%) with stage IIIa, and 34 (7%) with stage IIIb. The median tumor size was 27 mm (3-180 mm) and 131 patients had their tumor detected by screening mammography (most after 1992). For 92 patients, their carcinoma was not palpable and 211 (40%) had axillary lymph node metastases. Histology was invasive ductal in 87%, lobular in 7%, and 6% of tumors were classified as special types including tubular, medullary, and mucoid. Mastectomy was used as the initial definitive surgical procedure for 264 patients (50%) and breast conserving surgery for 226 (43%). The remaining patients underwent lesser procedures usually because of comorbidities or patient preference. Postoperative radiotherapy predominantly to the breast following breast conservation was employed in 216 patients (41%), adjuvant systemic chemotherapy postoperatively in 184 (35%), and postoperative Tamoxifen for 5 years was given to 295 patients (56%). Preoperative chemotherapy was used in 28 cases (5%).

PTHRP staining was detected in 79% of primary tumors. The relationship between standard prognostic and demographic factors is shown in Table 1. Patients with tumors expressing PTHrP were more likely to be postmenopausal and have smaller tumors that were ER and PR positive. Tumor grade was not associated with PTHrP status but the absence of lymphatic and/or vascular space invasion positive ER status and positive PR status were all significantly associated with positive PTHrP status.

**PTHRP staining and cancer survival.** Analysis of the initial data had yielded a hazard ratio of 0.52 (95% CI, 0.33-0.84; \( P = 0.007 \)) for patients with PTHrP-positive tumors. The corresponding hazard ratio estimate for the subsequent data was 0.37 (95% CI, 0.21-0.66; \( P = 0.001 \)) and the two estimates were consistent (\( P = 0.4 \); Table 2). A multivariate model adjusting for degree of nodal involvement, log of tumor size, PR and ER status, vascular-lymphatic invasion, and age was then fitted separately to both data sets. Age, ER status, and vascular-lymphatic invasion were removed from the model without
significant reduction in goodness of fit \( P = 0.3 \) (initial data), \( P = 0.06 \) (subsequent data). The resulting estimates of PTHrP hazard ratio differed only slightly from the univariate results, suggesting its action is largely independent (Table 3). Estimates from the two independent data sets were again consistent, so the data were combined to improve precision.

From the combined data, the estimated univariate hazard ratio for PTHrP staining was 0.43 (95% CI, 0.30-0.62; \( P < 0.001 \)) whereas the multivariate estimate was 0.47 (95% CI, 0.32-0.69; \( P < 0.001 \)). Hence, PTHrP was an independent predictor of outcome along with N stage, PR status, and log of tumor size. Again, it was found that age, ER status, and vascular-lymphatic invasion could be removed from the model (\( P = 0.4 \)). These results manifest in an estimated 10-year survival of 78% (95% CI, 72-81) for patients with PTHrP-positive tumors compared with 51% for PTHrP-negative patients (95% CI, 40-61; Fig. 1A). This difference was highly statistically significant (\( P < 0.001 \)).

Survival data of this patient group were determined for a number of prognostic factors: N stage, tumor size, axillary lymph node status, tumor grade, lymphatic/vascular space invasion, and ER and PR status. These results are found in Supplementary data.

### Table 1. Demographic details for 526 patients with stage I to III breast cancer (415 PTHrP-positive and 111 PTHrP-negative patients)

<table>
<thead>
<tr>
<th></th>
<th>All patients, ( n = 526 ) (%)</th>
<th>PTHrP positive, ( n = 415 ) (%)</th>
<th>PTHrP negative, ( n = 111 ) (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age (27-93)</strong></td>
<td>60</td>
<td>61</td>
<td>58</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Median tumor size, mm (3-180)</strong></td>
<td>27</td>
<td>27</td>
<td>31</td>
<td>0.007</td>
</tr>
</tbody>
</table>
| **Tumor stage**
  \( T_1a, T_1b \) (0-10 mm) | 54 (10)                          | 42 (10)                          | 12 (11)                          | 0.03        |
  \( T_1a, T_1b \) (>10-20 mm) | 204 (39)                         | 173 (42)                         | 31 (28)                          |             |
  \( T_2 \) (>20-50 mm) | 226 (43)                         | 172 (42)                         | 54 (49)                          |             |
  \( T_3 \) (>50 mm) / \( T_4 \) | 42 (8)                           | 28 (7)                           | 14 (13)                          |             |
| **Lymph node status**
  \( N_0 \) (0 nodes) | 315 (60)                          | 252 (61)                         | 63 (57)                          | 0.3         |
  \( N_1 \) (1-3 nodes) | 114 (22)                          | 93 (22)                          | 21 (19)                          |             |
  \( N_2 \) (4-9 nodes) | 68 (13)                           | 49 (12)                          | 19 (17)                          |             |
  \( N_3 \) (>9 nodes) | 29 (6)                            | 21 (5)                           | 8 (7)                            |             |
| **AJCC stage**
  I | 183 (35)                          | 158 (38)                         | 25 (22)                          | 0.01        |
  IIa | 161 (31)                          | 118 (28)                         | 43 (39)                          |             |
  IIb | 71 (14)                           | 59 (14)                          | 12 (11)                          |             |
  IIIa | 77 (15)                           | 55 (13)                          | 22 (19)                          |             |
  IIIb, IIIc | 34 (7)                           | 25 (6)                            | 9 (8)                            |             |
| **Menopausal status**
  Premenopausal | 97 (18)                           | 66 (16)                          | 31 (28)                          | 0.006       |
  Postmenopausal | 429 (82)                          | 349 (84)                         | 80 (72)                          |             |
| **ER status**
  Positive | 350 (68)                          | 290 (72)                         | 60 (54)                          | 0.01        |
  Negative | 165 (32)                          | 115 (28)                         | 50 (46)                          |             |
| **PR status**
  Positive | 312 (64)                          | 254 (66)                         | 58 (54)                          | 0.03        |
  Negative | 179 (36)                           | 130 (34)                         | 49 (46)                          |             |
| **Grade**
  1, well differentiated | 66 (13)                           | 55 (13)                           | 11 (10)                          | 0.5         |
  2, moderately differentiated | 270 (51)                           | 215 (42)                           | 55 (50)                          |             |
  3, poorly differentiated | 190 (36)                           | 145 (35)                           | 45 (40)                          |             |
| **Lymphatic/vascular invasion**
  Positive | 345 (66)                           | 275 (64)                          | 70 (67)                          | 0.6         |
  Negative | 175 (34)                           | 136 (36)                          | 39 (33)                          |             |
| **Tumor type**
  Invasive ductal | 458 (87)                           | 361 (87)                           | 97 (88)                          | 1.0         |
  Lobular | 35 (7)                            | 28 (7)                            | 7 (6)                            |             |
  Special types | 31 (6)                            | 25 (6)                            | 6 (6)                            |             |

Note: Tumor size, nodal status, and stage are all reported using the criteria of the current AJCC staging system. Positive ER and PR status defined as \( \geq 10\% \) of tumor cells staining for the receptor. Tumor grade was reported using the modified Bloom and Rich system. Special tumor types include good prognosis, low-grade lesions; tubular, medullary, and mucinous cancers.

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**PHTRP staining and bone metastases.** Kaplan-Meier survival curves were created for time until development of bone metastases (Fig. 1B). The fact that these curves diverge nonuniformly suggests nonapplicability of the Cox model, which seemed to result from dependence on tumor stage. After
stratification of the data by stage, there was no evidence of nonproportionality (Grambsch-Therneau, \( P = 0.96 \)). The estimated hazard ratio of bone metastases between patients with PTHrP-positive tumors and those with PTHrP-negative tumors, after stratification by stage, was 0.63 (95% CI, 0.41-0.98). Hence, the hazard ratio was lower in the PTHrP-positive group (\( P = 0.04 \)).

The appearance of metastases in major metastatic sites (e.g., bone, liver, lung, soft tissue, central nervous system (CNS), and locoregional sites) by PTHrP status is shown in Fig. 2. More metastases were observed in patients whose cancers were PTHrP negative. This applied to bone (22% versus 10%), liver (14% versus 6%), lung (22% versus 10%), soft tissue (16% versus 6%), and locoregional failure (22% versus 10%).

**Discussion**

We have investigated the importance for breast cancer behavior of PTHrP localization by immunohistochemistry in a consecutive series of patients at a single center. Our study of 526 patients for a median observation period of 10 years extends the analysis undertaken after a median observation of 5.6 years (13), confirms its findings, and enhances the statistical significance and precision. It establishes that positive PTHrP status in the primary breast cancers is independently associated with improved survival with reduced metastases to all tissue sites including bone. At the outset of the study, we had hypothesized that PTHrP production by primary breast cancers would increase the likelihood of development of skeletal complications and especially of bone metastases. Our finding, both in the initial and subsequent analyses, that patients with PTHrP-positive primary tumors had a significantly improved prognosis for survival was therefore a surprising one. This raises the possibility of effects of PTHrP on breast cancer behavior, which are distinct from its ability to promote bone resorption. It is the latter property that best explains a role for PTHrP in bone metastasis development and growth when it complements the general invasive properties of the cancer cells by conferring on them the ability to promote osteoclast formation and bone resorption.

The first indication that bone metastasis formation might be related to production of PTHrP by breast cancers came from a retrospective analysis of primary breast cancers and metastases in bone and soft tissues (9). Experimental evidence in support of a role for PTHrP in bone metastasis formation came from studies using human cancer cells that form lytic growths in nude mouse bone after intracardiac injection (6, 10–12). For example, nude mice injected with human breast cancer cells (MDA-MB-231) developed lytic bone deposits without any change in peripheral blood levels of PTHrP or calcium, but with elevated PTHrP levels in bone marrow plasma (11). Either inactivation with a neutralizing antibody of PTHrP in MDA-MB-231 cells (11) or inhibition by drug treatment of the activity of the PTHrP gene promoter (23) greatly reduced the ability of these cells to grow in bone after intracardiac injection. Furthermore, increased tumor growth in bone was achieved even with the less invasive MCF-7 cells when they were transfected to overexpress PTHrP (24). The latter cells were used also to show that tumor-derived PTHrP promoted osteoclast formation in host bone by enhancing

**Table 2. The persistence of the effect of PTHrP status on breast cancer survival in extended follow-up**

<table>
<thead>
<tr>
<th>Data</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.53</td>
<td>0.33-0.84</td>
<td>0.007</td>
</tr>
<tr>
<td>Subsequent</td>
<td>0.37</td>
<td>0.21-0.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Combined</td>
<td>0.43</td>
<td>0.30-0.62</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NOTE: Time to death was analyzed by Cox proportional hazards regression. The initial data relate to 367 patients followed for 1,896 person-years with 73 cancer deaths. The subsequent data relate to 429 women followed for 1,957 person-years with 128 cancer deaths. This included additional follow-up on 270 women from the initial data.

**Table 3. The estimated hazard ratio for PTHrP status, adjusted for possible confounders**

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Subsequent</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>( P )</td>
</tr>
<tr>
<td>( N_0 )</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>( N_1 )</td>
<td>2.03</td>
<td>1.07-3.87</td>
<td>0.03</td>
</tr>
<tr>
<td>( N_2 )</td>
<td>2.78</td>
<td>1.38-5.57</td>
<td>0.004</td>
</tr>
<tr>
<td>( N_3 )</td>
<td>9.36</td>
<td>4.47-19.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PR positive status</td>
<td>0.53</td>
<td>0.32-0.87</td>
<td>0.01</td>
</tr>
<tr>
<td>Log tumor size (mm)</td>
<td>2.31</td>
<td>1.50-3.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTHrP-positive staining</td>
<td>0.43</td>
<td>0.26-0.72</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NOTE: Analysis is presented for initial, subsequent, and combined data and includes factors found predictive of outcome. ER status, T stage, lymphatic-vascular space invasion, menopausal status, and tumor grade did not improve the model fit and were therefore not included.
production of receptor activator of nuclear factor κB ligand (RANKL; ref. 12).

The foregoing experimental evidence, suggesting a role for PTHrP in cancer establishment and growth in bone, illustrates the importance of osteoclast formation and activation in the bone metastasis process. To invade and grow in bone, as in any other tissue, cancer cells need general invasive properties that equip them to break down vessel walls, degrade connective tissue, and...
surgically removed and available for study. Weigelt et al. (33) were able to study eight such patients, with matched primary cancers and metastases, all of which were to tissues other than bone. They used gene expression profiling to show that in six of these eight cases, the metastases exhibited profiles very similar to the primary tumors. We were able to study 19 matched breast primary and bone metastasis samples from patients in the present study with surgery being carried out by one of the authors (P.M.F.C.) for pathologic fracture. The finding that bone metastases were PTHrP positive in six of the seven cases, in which the primary cancers were PTHrP negative whereas positive PTHrP staining was maintained in all the matched samples, is consistent with the suggestion that PTHrP status can be changed by the bone microenvironment in ways that can favor growth in bone.

There is increasing evidence for ways in which bone-derived growth factors, especially transforming growth factor β (TGF-β), can influence the cancer cell phenotype to enhance its growth in bone (6, 10, 34). Active TGF-β released from bone matrix during bone resorption provides a stimulus to PTHrP production by breast cancer cells. In support of this, expressing a dominant negative TGF-β receptor in MDA-MB-231 cells led to substantially reduced tumor establishment and growth in bone after intracardiac injection of the cells into nude mice (10). A further potential contribution from TGF-β locally comes from its ability to enhance RANKL-induced osteoclast formation (35), thereby potentiating the PTHrP effect. Furthermore, elevating the ambient calcium levels significantly enhanced PTHrP production by human breast cancer (MCF 7) cells in vitro, as well as amplified the TGF-β-induced elevation of PTHrP (36). The cooperative effects of PTHrP and TGF-β compose a means of amplifying local events in bone in favor of tumor growth in that site, illustrating the major influence on cancer behavior of the bone microenvironment (6).

It remains to be determined whether PTHrP might indeed confer on cancer cells a less invasive phenotype, and possible mechanisms are being explored. PTHrP is a multifunctional protein (37, 38) with biological activities ascribed to several domains of the molecule apart from the parathyroid hormone–like NH₂-terminal region that mediates the bone-resorptive effect. The prospect of PTHrP being protective at one stage of cancer and having a deleterious role at another is a credible one, given that its role in bone metastasis formation is essentially to provide the specific ability to promote bone resorption, thereby complementing the general invasive properties of the cancer cells, and further, that PTHrP production can readily be enhanced by local conditions in bone. Another example of a protein with a divergent effect in cancer is TGF-β, which acts early as a tumor suppressor by inhibiting proliferation of epithelial, endothelial, and hemopoietic cells. Refractoriness to these effects develops later and overexpression of TGF-β leads to a microenvironment conducive to tumor growth (reviewed in refs. 39–41).

A role for PTHrP in breast cancer biology may not be surprising in view of the evidence that it is essential for formation of the mammary gland. In PTHrP−/− mice rescued by transgenic expression of PTHrP in cartilage, mammary development begins but branching morphogenesis fails (42). In both mouse and human breast, PTHrP is expressed in the epithelial cells and the receptor (PTH1R) in mesenchymal cells (38, 42, 43). A recently recognized paracrine action of PTHrP in the developing breast is its promotion of RANKL by mammary epithelial cells, a response to promote angiogenesis. Specific requirements in bone are the ability to adhere to that tissue and, most importantly, to promote the formation of active osteoclasts from precursors in host bone, thereby initiating resorption and allowing the tumor to establish and expand (4–6). Relevant to this, when gene array studies have been applied to human breast cancer cells of strong and weak bone-metastasizing ability, it is noteworthy that there is a predominance of gene products that favor either promotion of osteoclast formation or the adherence of cells to bone. Examples are the appearance in gene array studies, on the one hand, of interleukin (IL)-11, matrix metalloproteinase 1 (25), and IL-8 (26) as stimulators of osteoclasts, and, on the other hand, of osteopontin, connective tissue growth factor, and CXCR4 (25, 27) as factors favoring the homing and/or adherence of cancer cells to bone. Thus, although PTHrP has been extensively investigated, it is clear that there are other breast cancer products that could profoundly influence bone metastasis establishment by promoting osteoclast formation, including prostaglandins, II-6, IL-8 (26), and macrophage colony-stimulating factor (28).

The results described here seem to be at variance with a number of reports, including our own, suggesting that PTHrP staining in primary breast cancers is associated with the development of bone metastases (29–32). These studies can be questioned from a number of points of view, including small numbers, case selection, advanced disease, limited follow-up, and retrospective accrual. This report describes the only long-term prospective study of unselected patients. Its major finding that PTHrP-positive tumors were independently predictive of improved patient survival, with reduced metastases at all sites, questioned from a number of points of view, including small numbers, case selection, advanced disease, limited follow-up, and inconsistent with the suggestion that PTHrP status can be changed by the bone microenvironment in ways that can favor growth in bone.

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shown also to prolactin, which might act indirectly through its induction of PTHrP. Mice rendered null for RANKL show lack of development of lobulo-alveolar structures during pregnancy despite earlier stages of mammary gland development being normal (44, 45).

Studies with primary breast cancers have identified a set of genes associated with poor prognosis (46, 47), predicting development of metastases and decreased survival. A similar genetic “signature” has been noted in MDA-MB-231 human breast cancer cells, subpopulations of which display a genetic profile predicting the site of metastases, particularly those growing in bones of the host nude mice (25, 48). Effects of the bone microenvironment on tumor cell behavior underline how important it is in clinical management to use bone-targeted drugs in ways which appropriately complement specific antitumor therapies.

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References

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