Soluble ErbB3 Levels in Bone Marrow and Plasma of Men with Prostate Cancer

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Abstract

Purpose: Prostate cancer tends to metastasize to bone and induce osteoblastic lesions. We identified a soluble form of ErbB3 (sErbB3), p45-sErbB3, in bone marrow supernatant from men with prostate cancer bone metastasis and showed that p45-sErbB3 enhances bone formation. We aimed to understand clinical implications of sErbB3 by establishing an ELISA to detect sErbB3 levels in bone marrow and plasma samples.

Experimental Design: We did ELISAs on marrow from 108 men [34 with androgen-dependent disease, 30 with androgen-independent disease (AI) but negative bone scan (AI/BS-), and 44 with AI and positive bone scan (AI/BS+)], sequential marrow from 5 men during treatment, plasma from 52 men before and after docetaxel treatment, and plasma from 95 men ages ≥70 years old without prostate cancer.

Results: Some men with clinically detectable bone metastasis had high sErbB3 levels. Within the AI/BS- group, higher sErbB3 levels seemed to yield lower rates of bone metastasis. In the AI/BS+ group, detectable bone metastases took longer to appear in men with higher sErbB3 levels than in men with lower sErbB3 levels (median, 82 versus 41 months). However, high sErbB3 levels did not confer survival benefit after metastasis development. Among men with metastatic progression in bone, docetaxel treatment reduced plasma sErbB3 (P < 0.0001) but did not affect bone-specific alkaline phosphatase (P = 0.206) or prostate-specific antigen (P = 0.906). sErbB3 was also detected in men without prostate cancer.

Conclusions: The apparent correlation between higher sErbB3 levels and longer time to bone metastasis suggests that sErbB3 participates in progression in bone of prostate cancer.

Advanced prostate cancer often metastasizes to distant sites, most commonly bone (1). Bone metastases from prostate cancer are primarily osteoblastic, with an overall increase in bone formation (1). In contrast, bone metastases from lung, kidney, or breast are frequently osteolytic (2). The effect of osteoblastic reaction on the progression of prostate cancer in bone is not clear. Interestingly, metastases of prostate cancer seem to progress more slowly than do osteolytic metastases associated with other tumors. Nevertheless, development of bone metastases confers poor prognosis (3), and no effective strategy has been found to prolong survival among men with metastatic prostate cancer at this stage.

Despite similarities in clinical presentation, a recent study of the pathologic, immunologic, and molecular features of specimens of prostate cancer bone metastases at autopsy showed that metastatic hormone-refractory prostate cancer is actually a heterogeneous group of diseases (4). Findings from that study further suggested that different foci of metastatic disease within the same individual could vary in immunophenotype and genotype (4). These observations underscore the difficulty in identifying biomarkers for the early detection of bone metastasis and in designing strategies to treat or prevent the progression of disease. Understanding the mechanisms of prostate cancer progression within the bone compartment, and identifying the molecular pathways involved, will help to identify men at high risk of developing metastases and will also serve as a basis for new diagnostic, therapeutic, and possibly preventive modalities for metastatic disease.

The propensity of prostate cancer cells to metastasize to bone and the osteoblastic nature of most bone lesions imply that bidirectional interactions exist between the cancer cells and the bone microenvironment (5–7). Putative factors secreted by prostate cancer cells that affect osteoblast growth and differentiation could be involved in the progression of prostate cancer in bone (8). Several candidate factors including endothelin-1...
advanced prostate cancer. Levels in bone marrow and plasma samples from men with prostate cancer and bone metastasis (13). Immunohistochemical analysis of tissue specimens showed that soluble forms of ErbB3 (sErbB3) were expressed by prostate cancer cells in lymph nodes and bone marrow but not by cells in the prostate (13). Receptor-binding studies showed that p45-sErbB3 binds specifically to membranes prepared from cells of osteoblast lineage but not membranes from prostate cancer cells (14), suggesting that p45-sErbB3 could affect osteoblasts. Further functional studies suggest that p45-sErbB3 has osteoblast regulatory activity (15). Collectively, these findings indicate that sErbB3s could be involved in the bone-forming phenotype typical of bone metastases from prostate cancer. In this study, we sought to understand the clinical implications of the presence of sErbB3 by establishing an ELISA method to allow us to measure sErbB3 levels in bone marrow and plasma samples from men with advanced prostate cancer.

**Patient Specimens and Methods**

**ELISA for sErbB3.** A commercially available ELISA kit [human ErbB3 DuoSet (DY348); R&D Systems] could not detect recombinant p45-sErbB3, perhaps because p45-sErbB3 contains only the first half of the extracellular domain of ErbB3. We therefore modified that assay by using one of the monoclonal antibodies to capture the sErbB3 from the study samples and subsequently using a polyclonal antibody against the entire extracellular domain of ErbB3 to detect the protein in supernatant fractions of bone marrow and plasma samples. The sErbB3 was captured onto 96-well plates for ELISA. First, 100 μL (4 μg/mL) of the capture monoclonal antibody MAB3481 (R&D Systems) were added to each well of a 96-well plate and incubated overnight at room temperature. Wells were then washed with PBS plus 0.1% Triton X-100 and incubated with PBS containing 1% bovine serum albumin to block nonspecific binding of the antibodies. Then 100 μL of the supernatant from bone marrow or plasma samples (prepared as described below) were added to each well, and the plates were incubated at room temperature for 2 h. A polyclonal goat anti-ErbB3 antibody (1 pg/μL; R&D Systems) was added and the plates were incubated for another 2 h. The sErbB3 was then detected with horseradish peroxidase–conjugated anti-goat antibody (SC2020; Santa Cruz Biotechnology) and a substrate reagent pack (DY999; R&D Systems). The standard used in these assays was recombinant p45-sErbB3, expressed by PC-3 prostate cancer cells infected with recombinant p45-sErbB3 adenovirus (Ad-p45), and purified from the conditioned medium by metal affinity chromatography (15); standards were run in parallel in every assay.

**Bone marrow samples.** Samples were collected according to a protocol approved by the institutional review board of M. D. Anderson Cancer Center. Marrow samples were collected by aspiration of the iliac crests. About 10 mL of marrow was drawn into a syringe containing 1 mL of sodium heparin (1,000 USP units/mL) and were centrifuged at 4°C for 20 min at 2,500 rpm, and the supernatants were transferred into cryotubes (Sarstedt). All samples were processed the day they were drawn and frozen at -85°C.

**Patients.** Because p45-sErbB3 had originally been identified in bone marrow sample supernatants, we used our ELISA to assess sErbB3 levels in bone marrow samples collected from 108 men between May 1993 and September 2002. To see if sErbB3 levels were associated with disease stage, we grouped these men according to their clinical status at the time of the bone marrow biopsy: group 1 was 34 men with androgen-dependent (AD) disease, i.e., men who had not received hormonal ablation therapy or whose disease was responding to such treatment at the time of the biopsy; some of these men had had prostatectomy or radiation therapy. Group 2 comprised 30 men with androgen-independent (AI) disease that did not respond to hormonal ablation therapy but had no bone metastases. These men (AI/BS-) had elevated serum prostate-specific antigen (PSA) levels after hormone ablation therapy or orchietomy (testosterone levels confirmed as <50 ng/dL) but had no evidence of bone metastasis on bone scans obtained at the time of biopsy (some of these men may have had metastatic disease elsewhere, e.g., in soft tissue, lymph nodes, lung, or liver). The third group consisted of 44 men with AI disease who had bone metastases at the time of biopsy. These men (AI/BS+) had elevated serum PSA levels after androgen ablation therapy or orchietomy (testosterone levels confirmed as <50 ng/dL) and had positive bone scans on the same day were tested for N-telopeptide. The PSA, AP, and N-telopeptide measurements were part of the study design.

**Plasma samples from men treated with docetaxel.** We also obtained plasma samples from 52 men enrolled in a clinical trial of docetaxel for the treatment of bone metastases from prostate cancer (16). Those samples were obtained before and after one 6-wk period of docetaxel treatment (30 mg/m² on days 1, 8, 15, and 22). Plasma samples were drawn on days 1 and 43 and tested for sErbB3, bone-specific alkaline phosphatase (AP), and PSA levels; urine samples collected on the same days were tested for N-telopeptide. The PSA, AP, and N-telopeptide measurements were part of the study design.

**Plasma samples from men without prostate cancer.** Plasma samples from men ages 70 years or older, with no evidence of prostate cancer or bone-related disease [i.e., Paget’s disease, fresh fractures of bone not related to cancer], or osteopoikilosis were obtained from TexGen, a collaborative genetic research effort of the Texas Medical Center.

**Statistical analyses.** Fisher’s exact test or χ² tests were used to assess associations between categorical variables. Kruskal-Wallis rank analysis was used to examine the difference in sErbB3 levels among the AD, AI/BS-, and AI/BS+ groups, and Wilcoxon’s rank-sum test was used to assess differences in sErbB3 levels between two disease groups. Time-to-disease progression and survival curves were estimated with the Kaplan-Meier method. The multivariate Cox proportional hazards regression model was used to examine risk factors related to time-to-disease progression after adjusting for other factors. Two-sided log-rank tests were used to assess differences in time to events between groups. P values of <0.05 were considered statistically significant. Statistical analyses were done with S-Plus version 2000 (Insightful Corp.). We also set the 95th percentile of the mean sErbB3 values in the AD group as a threshold value and compared the proportions of men in the AI/BS- and AI/BS+ groups whose average sErbB3 levels were above this threshold by using Fisher’s exact test. We applied a cube-root transformation to the sErbB3 measurement values and used normal-mixture models to test for possible heterogeneity in the sErbB3 level measurements. sErbB3 measurements were modeled by using a finite mixture model with G components (17), assuming that

\[ \text{Likelihood (model parameters|data)} = \pi_1 f_1 (\text{data}|\theta_1) + \pi_2 f_2 (\text{data}|\theta_2) \]

where \( \pi_1 \) and \( \theta_1 \) are the density and parameters of the \( k \)th component in the mixture and \( \pi_k \) is the probability that an observation belongs to the \( k \)th component. We assumed that \( f_k \) is the normal (Gaussian) density, parameterized by its mean \( \mu_k \) and SD \( \sigma_k \).

The expectation-maximization algorithm is a general approach for parameter estimation in the mixture model. To select a model in which G components best fit a particular population, we used Bayesian model selection with Bayes factors and posterior model probabilities, implemented by the Bayesian information criterion. According to this criterion, the model with the smallest Bayesian information criterion should be considered the optimal one. The R software package MCLUST/EMCLUST\(^*\) was used for all mixture model calculations.

Results

An ELISA for sErbB3. We established an ELISA to measure sErbB3 levels in bone marrow supernatants and plasma. In addition to p45-sErbB3, transcripts of various lengths of extracellular domain of sErbB3 have been reported (18). Although it is not clear whether all of these transcripts are translated into proteins, the ELISA protocol used was expected to detect the various forms of sErbB3 likely to be present in the samples. The sensitivity of detection in the colorimetric method is ~0.5 ng p45-sErbB3 per mL (data not shown). The presence of serum in the protein standards did not affect the linearity or sensitivity of the standard curve (data not shown).

Bone marrow sErbB3 levels at different disease stages. ELISA revealed wide ranges of sErbB3 levels in all three groups of men with prostate cancer (Fig. 1A). In the AD group, sErbB3 concentrations ranged from 0 (undetectable) to 4.45 ng/mL, with a median of 1.32 ng/mL and a mean (±SD) of 1.33 ± 0.86 ng/mL. In the AI/BS- group, sErbB3 concentrations ranged from 0.06 to 2.55 ng/mL (median, 0.78 ng/mL; mean, 1.01 ± 0.73 ng/mL). In the AI/BS+ group, sErbB3 levels ranged from 0.04 to 11.76 ng/mL (median, 0.93 ng/mL; mean, 1.71 ± 2.38 ng/mL). The large SD in this third group came from three men who had very high sErbB3 levels, as discussed below.

To examine whether sErbB3 levels were different among the three groups, we applied cube-root transformation to all the data and used Kruskall-Wallis rank test to compare the three groups. sErbB3 levels were not different among the three groups (P = 0.274). Comparing the groups with each other with a Wilcoxon rank-sum test also showed no difference between the groups [AD versus AI/BS-, P = 0.1035; AD versus AI/BS+, P = 0.522; AI/BS- versus AI/BS+, P = 0.3163; and AD versus (AI/BS- plus AI/BS+), P = 0.221].

We then compared the three groups according to the number of men whose sErbB3 levels were above the 95th percentile of sErbB3 levels in the AD group, which was 2.73 ng/mL. Two (6%) of the 34 men in the AD group had sErbB3 levels higher than 2.73 ng/mL, whereas 0 of 30 in the AI/BS- group and 5 (11%) of 44 in the AI/BS+ group had such levels. This comparison revealed a marginally significant difference between the AI/BS- and AI/BS+ groups (P = 0.076, Fisher’s exact test).

Because sErbB3 levels in some of the AI/BS+ group members were much higher than those of others, we next tested for the presence of different subsets in the same group. After the cube-root transformation of all of the values, we applied the normal-mixture model to each of the three groups and found that sErbB3 measurements in the AD group generated one population centered near 1.04 ± 0.28, and those in the AI/BS- group generated one population centered at 0.94 ± 0.26 (Fig. 1B). In contrast, sErbB3 measurements for the AI/BS+ group followed a mixture distribution of two densities, one...
centered near 0.97 ± 0.28 and another near 2.12 ± 0.15 (Fig. 1B). The second peak comprised three men with very high sErbB3 levels (mean, 9.54 ± 2.01 ng/mL); by comparison, the mean for the first group was 0.924 ± 0.786 ng/mL. These analyses suggest that a subgroup of men with clinically detectable bone metastasis have high levels of sErbB3 in their bone marrow supernatants.

Of the 30 men in the AI/BS- group, 21 subsequently developed bone metastases. The mean sErbB3 level in these 21 men was 0.80 ± 0.58 ng/mL. The remaining nine men (Fig. 1A, black bars) never developed bone metastasis. Interestingly, those 9 men had higher mean sErbB3 levels (1.50 ± 0.83 ng/mL, Wilcoxon rank-sum test; P = 0.037).

We next analyzed whether the men in the AI/BS+ group showed differences in time to progression according to sErbB3 level. Time-to-progression was defined as the time from the initial diagnosis of prostate cancer to the time of the positive bone scan. We then divided the group into three parts as low, medium, and high sErbB3 level based on the quartiles of sErbB3, which corresponds to the first quartile, middle 50%, and last quartile, respectively. The clinical characteristics of these three groups of patients are summarized in Table 1. Statistical analyses by comparing the three sErbB3 groups and clinical characteristics using Kruskall-Wallis rank analysis (for age, PSA, Gleason score) and \( \chi^2 \) test (for prostatectomy and radiotherapy) are shown in Table 1. No statistically significant imbalances were found among the three sErbB3 groups with any of these clinical variables. Median time-to-progression in the first and last quartile subgroups were 41 and 82 months, respectively (\( P = 0.008 \), log-rank test; Fig. 2A). The time to progression for the middle 50% subgroup was intermediate between the quartile subsets (43 months), and the difference in time to progression became marginally significant when the three groups were compared (\( P = 0.0699 \), log-rank test; Fig. 2B). These findings suggest that higher sErbB3 levels are associated with a longer metastasis-free interval. We further evaluated sErbB3 levels and time-to-progression with a univariate Cox model and confirmed that men with higher sErbB3 levels were at lower risk of developing bone metastasis compared with low sErbB3 levels (relative risk, 0.35; \( P = 0.026 \)), but survival time (defined as the time from the positive bone scan to death) was not different between the low and high sErbB3 groups (\( P = 0.453 \), log-rank test; Fig. 2C). Age is statistically significant (hazard ratio, 1.05; \( P = 0.045 \)) in time-to-progression univariate Cox regression analysis. However, Gleason score (\( P = 0.499 \)), prostatectomy (\( P = 0.992 \)), and radiotherapy (\( P = 0.377 \)) were not significant in the univariate Cox regression analysis.

Multivariate Cox regression model was also fitted to examine sErbB3 levels related to time-to-progression after adjusted for other factors. A stepwise variable selection was done and sErbB3 and age were in the final model. The hazard ratio is 0.389 (\( P = 0.04 \)) for high sErbB3 in comparison with low sErbB3, after adjusting for age.

**Changes in sErbB3 levels over the course of the disease.** As reported above, sErbB3 levels varied widely among men in the AI/BS+ group; moreover, no correlations were found between sErbB3 levels and levels of bone-specific AP (an osteoblast-activation marker) or PSA (a tumor-size marker; data not shown), probably because of the heterogeneity of disease among these men (4). We thus attempted to determine whether sErbB3 levels correlated with AP or PSA levels over time in the five men from whom at least three sequential bone marrow specimens were available and who had detectable levels of sErbB3. Changes in the levels of sErbB3, PSA, and AP from the same men were compared over time. Levels of sErbB3 remained relatively similar throughout the course of disease in three men (patients 1, 2, and 4; Fig. 3). sErbB3 levels remained elevated after 6 years of follow-up in patient 1, and after 3 years of follow-up in patient 2. In these two men, AP levels were also relatively constant, whereas PSA values increased over time in all of the samples. As for patients 3 and 5, increases in sErbB3 levels were observed after 3 and 4 years of follow-up. These two men also showed increases in PSA and AP levels. The small size of this sample precludes definite conclusions regarding correlations between sErbB3 levels and either PSA or AP levels; however, these data seem to suggest that changes in sErbB3 levels were comparable with changes in AP levels in all five men, whereas changes in sErbB3 levels did not consistently correlate with PSA levels. The possible correlation between sErbB3 and AP, an osteoblast marker, suggests that sErbB3 level may reflect the bone aspect of prostate cancer metastasis rather than the proliferation of prostate cancer cells.

Because there is no standard treatment for prostate cancer with bone metastasis, these five men had been given different treatment regimens. A decrease in PSA levels by >50% from baseline was considered to have a response to treatment. We evaluated the effect of the treatments received on bone marrow sErbB3 levels and found that there were no consistent changes in either sErbB3 or AP levels in response to treatment.

**sErbB3 levels in bone marrow versus plasma in individual men.** Because bone metastases develop within the bone marrow cavity, bone marrow supernatant may be more relevant than blood for studying factors that participate in bone metastases.

### Table 1. Clinical characteristics of patients in AI/BS+ group

<table>
<thead>
<tr>
<th>sErbB3 levels</th>
<th>Age (median, range)</th>
<th>PSA level (median, range)</th>
<th>Gleason score (median, range)</th>
<th>Prostatectomy</th>
<th>Radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (n=11)</td>
<td>61 (54-77)</td>
<td>122 (2.4-550)</td>
<td>8 (6-10)</td>
<td>3/11 (27%)</td>
<td>4/11 (36%)</td>
</tr>
<tr>
<td>Medium (n=22)</td>
<td>60 (47-76)</td>
<td>97 (0.7-5,424)</td>
<td>8 (7-10)</td>
<td>9/20 (45%)*</td>
<td>10/19 (53%)*</td>
</tr>
<tr>
<td>High (n=11)</td>
<td>66 (49-71)</td>
<td>90 (4-8-2,737)</td>
<td>8 (7-10)</td>
<td>3/11 (27%)</td>
<td>8/11 (73%)</td>
</tr>
<tr>
<td>P value</td>
<td>0.623*</td>
<td>0.818*</td>
<td>0.707*</td>
<td>0.488*</td>
<td>0.23*</td>
</tr>
</tbody>
</table>

*Information was not available for a few patients.

† Kruskall-Wallis rank analysis.

‡ \( \chi^2 \) test.
metastasis. However, blood samples are much easier to obtain and more practical for tests that must be repeated over time. We thus searched the Bone Marrow/Serum Bank of M. D. Anderson’s Prostate Cancer Specialized Programs of Research Excellence Specimen Core for cases in which bone marrow and plasma samples had been collected from the same patient on the same day to allow us to compare sErbB3 levels in bone marrow supernatant to sErbB3 levels in plasma samples. In the five bone marrow/plasma pairs identified and tested, the plasma samples contained less sErbB3 than did the bone marrow samples (81.6% $\pm$ 8.6% of bone marrow levels). However, the levels in the marrow samples paralleled those in the plasma samples. These results suggest that plasma sErbB3 levels may correlate with levels in bone marrow supernatant samples and thus may be a valuable surrogate for measuring sErbB3 levels, especially over time.

**Plasma sErbB3 levels before and after docetaxel treatment.** We next sought to compare changes in plasma sErbB3 levels with changes in plasma PSA and bone-specific AP and urinary N-telopeptide (a marker of osteolytic activity) before and after treatment with the taxane docetaxel. We measured sErbB3 in blood samples from 52 men participating in a clinical trial at M. D. Anderson Cancer Center of docetaxel for prostate cancer and bone metastasis (16). Interestingly, 2 of these 52 men had elevated sErbB3 levels (7.52 and 13.04 ng/mL) before treatment, a finding consistent with our earlier discovery of a subgroup of men in the AI/BS+ group with high sErbB3 levels. After treatment, sErbB3 levels were significantly lower than those before treatment (Table 2; $P < 0.0001$, Wilcoxon signed-rank test for paired data). However, the change of plasma sErbB3 after a 6-week docetaxel treatment is not significantly associated with survival ($P = 0.724$ in a Cox model). Urinary N-telopeptide and plasma bone–specific AP levels also seemed to be lower after treatment than before, but these apparent differences were not statistically significant. An apparent increase in PSA over time was also not statistically significant.

**Plasma sErbB3 levels in men without prostate cancer.** We also examined sErbB3 levels in plasma samples from 95 men ages $\geq 70$ years with no evidence of prostate cancer (who may or may not have been treated for other diseases unrelated to bone). The sErbB3 levels in these samples ranged from 0 (undetectable) to 6.9 ng/mL (median, 0.15 ng/mL; mean, 0.6 $\pm$ 1.2 ng/mL; Fig. 4). Thus sErbB3 can be present, at a wide range of concentrations, among men with no evidence of prostate cancer, suggesting that plasma sErbB3 levels may not be related solely to prostate cancer but may also be involved in other yet-unknown physiologic or pathologic conditions.

**Discussion**

Our previous discovery that p45-sErbB3 has osteoblast regulatory activity (15) led us to search for its clinical implications by establishing an ELISA to measure sErbB3 levels in men with prostate cancer at different stages. Although the current ELISA cannot specifically detect p45-sErbB3, we could detect secreted forms of ErbB3 in bone marrow and plasma samples, and sErbB3 levels were greatly elevated in a subgroup of men with prostate cancer and bone metastasis. Moreover, among men with AI disease, those with higher sErbB3 levels had slower progression to metastasis than those with lower sErbB3 levels. Other notable findings were that changes in bone marrow sErbB3 levels during disease progression over time may correlate better with serum AP levels than with serum PSA levels; that sErbB3—but not PSA, bone-specific AP levels, or N-telopeptide levels—decreased after a single cycle of docetaxel treatment; and that sErbB3 was detected in plasma samples from men ages $\geq 70$ years with no clinical evidence of prostate cancer.
Our observation that only a subgroup of men with bone metastases had elevated sErbB3 levels supports the concept of clinical and biological heterogeneity in prostate cancer bone metastases (4). This heterogeneity may be one reason for the general ineffectiveness of therapies targeting specific factors, e.g., endothelin-1 (19). The fact that only a subset of men responded to atrasentan treatment suggests that endothelin-1 is one of many paracrine factors involved in the interactions between osteoblasts and prostate cancer cells. Similarly, sErbB3 may play a role in the bone metastasis of a subgroup of patients.

Within the AI/BS- group, men with higher sErbB3 levels seemed to have lower rates of bone metastasis. Similarly, in the AI/BS+ group, clinically detectable bone metastases took longer to appear in men with higher sErbB3 levels (median, 82 months) than in men with lower sErbB3 levels (median, 41 months). However, high levels of sErbB3 did not seem to

### Table 2. Changes in sErbB3 levels after docetaxel treatment

<table>
<thead>
<tr>
<th>Factor</th>
<th>Baseline levels</th>
<th>Cycle 2 levels</th>
<th>Wilcoxon test *</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>N</td>
</tr>
<tr>
<td>Plasma sErbB3 †</td>
<td>52</td>
<td>1.065 (1.989)</td>
<td>47</td>
</tr>
<tr>
<td>Plasma bone-specific AP</td>
<td>51</td>
<td>59.496 (80.142)</td>
<td>46</td>
</tr>
<tr>
<td>Urinary NTX</td>
<td>50</td>
<td>61.12 (45.114)</td>
<td>45</td>
</tr>
<tr>
<td>Plasma PSA</td>
<td>52</td>
<td>141.034 (276.933)</td>
<td>45</td>
</tr>
</tbody>
</table>

Abbreviation: NTX, N-telopeptide.
*Wilcoxon signed-rank test for paired data.
†Change of Plasma sErbB3 from C2D1 to baseline was not significantly associated with survival (P = 0.724 in a Cox model).
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Fig. 4. Distribution of sErbB3 levels in plasma samples from men without prostate cancer. Samples are ordered from the lowest to highest sErbB3 levels from left to right along the x axis.

Confer a survival benefit after the development of metastasis (Fig. 2C). Perhaps not surprisingly given the complexity of prostate cancer bone metastasis, it is not possible to provide a clear explanation for the observed difference in time to progression. Because we showed that p45-sErbB3, a form of sErbB3, can induce the formation of new bone (15), we hypothesize that an increase in the secretion of sErbB3 by prostate cancer cells may induce increased bone volume at the metastatic sites. Such increases in bone volume could limit the space available to cancer cells and hence help in confining tumor expansion. This could explain why metastases of prostate cancer seem to progress more slowly than do osteolytic metastases associated with other tumors. However, ongoing increases in numbers of osteoblasts and prostate cancer cells during disease progression ultimately lead to the activation of osteoclasts via RANKL and other paracrine factors and to increased osteolysis (20, 21). Bone resorption then facilitates tumor cell growth by creating gaps and releasing growth factors contained in the bone matrix (22). The osteoclastic stage of metastasis signals rapid disease progression and, ultimately, short survival time. Testing these hypotheses, however, will require further investigation.

Growth of prostate cancer cells in bone involves interactions between the cancer cells and other types of cells in the bone microenvironment (23). Both osteoblast activity (indicated by bone-specific AP) and osteoclast activity (indicated by N-telopeptide) are elevated in men with prostate cancer and bone metastases (24, 25). Histomorphometric quantification also consistently shows changes in both bone formation and resorption within metastatic foci in iliac crest biopsy samples (26). In the current study, changes in sErbB3 levels correlated with changes in AP levels, but not with PSA levels, in longitudinal samples from men with prostate cancer and bone metastases. Although too few men were tested for definitive analysis, the presence of a correlation between sErbB3 levels and AP levels would be consistent with our observation that sErbB3 is expressed in both activated osteoblasts and prostate cancer cells (13) and that sErbB3 has osteoblast-regulatory activity (15). Such a correlation would also implicate sErbB3 in the interactions between prostate cancer cells and osteoblasts.

In our study, receipt of a 6-week period treatment of docetaxel led to decreases in sErbB3 levels. Docetaxel is also used in the treatment of breast cancer (27) and non–small cell lung cancer (28), among others. In addition to affecting prostate cancer cells, docetaxel may also affect osteoblasts, osteoclasts, or other stromal cells. Interestingly, only sErbB3, which probably is secreted by both prostate cancer cells and osteoblasts (13), showed declines after docetaxel, whereas bone-specific AP and PSA did not. However, decreases of PSA and AP levels were detected in samples collected at later time points (data not shown). Whether the delayed response of these two markers reflects the stability of these proteins is unknown.

Our observation that sErbB3 was detected in men with no evidence of prostate cancer suggests that sErbB3 in the bone marrow or plasma might not be related solely to prostate cancer. Indeed, transcripts encoding various secreted forms of ErbB3 have been detected in normal human tissues, including colon, kidney, placenta, and liver as well as in cell lines derived from ovarian carcinoma (18). Interestingly, Lee et al. (29) also reported that p85-sErbB3, but not p45-sErbB3, inhibited heregulin-stimulated ErbB2, ErbB3, and ErbB4 activation, probably by competing with heregulin binding to the ErbB3 or ErbB4 receptors. Collectively, these observations suggest that sErbB3 may also be involved in yet-unknown physiologic or pathologic conditions other than prostate cancer.

In summary, the proclivity of prostate cancer cells to metastasize to bone and the stimulation of prostate cancer cell growth by osteoblasts (6) suggests that the survival and growth of prostate cancer cells in the bone environment depends on factors produced by bone (5, 7, 8). At present, no effective way has been found to inhibit the bone-forming activity induced by prostate cancer bone metastasis. Thus, the role of increased bone formation in the clinical progression of prostate cancer in bone is still not clear. Further exploration of the relationship between the expression of osteoblast-stimulatory factors and the prostate cancer progression in bone will improve our ability to identify patients who would benefit from preventative or bone-targeted therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

5. Gleaves ME, Hsieh J-Y, von Eschenbach AC, Chung LWK. Prostate and bone fibroblasts induce human...