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Review Article

Molecular Evidence-Based Use of Bone Resorption-Targeted Therapy in Prostate Cancer Patients at High Risk for Bone Involvement

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Abstract

Background: To improve median survival of patients with prostate cancer that has metastasized to bone, we need to better understand the early events of the metastatic process in skeleton and develop molecular tools capable of detecting the early tumor cell dissemination into bones (micrometastasis stage). However, the initial phase of tumor cell dissemination into the bone marrow is promptly followed by the migration of tumor cells into bone matrix, which is a crucial step that signals the transformation of micrometastasis to macrometastasis stage and clinically evident metastasis. The migration of cancer cells into bone matrix requires the activation of local bone resorption. Such an event contributes to tumor cell hiding/escaping from high immunologic surveillance of bone marrow cells. Within bone matrix, tumor cells are establishing plethoric cell–cell interactions with bone marrow-residing cells, ensuring their survival and growth. Recently, RT-PCR detections of tumor marker transcripts, such as PSA and PSMA mRNA performed in RNA extracts of peripheral blood nucleated cells and bone marrow biopsy, have enabled the stratification of patients with clinically localized prostate cancer being of high risk for extraprostatic disease and bone involvement. Therefore, it is conceivable that bisphosphonate blockade of bone resorption can inhibit the migration of tumor cells into bone matrix during the early phase of disease dissemination into bone marrow (micrometastasis stage). Consequently, assessment of the efficacy and efficiency of bisphosphonates to arrest the evolution of bone lesions in this particular clinical setting of patients with clinically localized prostate cancer and positive molecular staging status (high risk for bone involvement) is warranted.

Introduction

The most important clinical manifestation of cancer, which defines treatment strategy, disease prognosis and overall survival, is tumor cell dissemination (metastasis) into organs distant from the primary tumor (1). The process of metastasis implicates a cascade of events, involving angiogenesis, detachment from the primary tumor (cell mobility), migration into the adjacent tissue (invasion), adhesion onto the wall and entry into the local vessels, dissemination through the systemic circulation, survival in peripheral blood, extravasations, attachment onto specific organs (seed and soil theory), and local ectopic/metastatic growth in host tissue (1–3).

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There are serious concerns stemming from the fact that bones correspond to the most prevalent site of prostate cancer metastases, producing mainly, but not exclusively, the osteoblastic reaction of skeletal tissue (3–7). That is because the actual number of the metastatic foci into bone is the single and the most powerful adverse prognostic factor that predetermines, sooner or later, lethal outcome (8–10). In addition, the extension of skeletal lesions (>6 foci) correlates strongly with limited response to androgen ablation therapy; bony lesions are, almost always, the exclusive sites of disease progression to hormone refractory stage (11,12). Notably, disease progression to hormone refractory stage in bones develops while androgen ablation therapy provides still complete control of disease at the primary site (9,10,13,14). Apparently, bone constitutes a favorable microenvironment of extreme biologic importance facilitating the development of hormone- and chemotherapy-refractory tumor biology (5–7,9,10).

The altered tumor cell biology in bone metastasis is secondary to interactions of prostate cancer cells with bone cells, which can promote both the growth and survival of metastatic cancer cells locally (2,7,9–11). Among bone-derived growth factors abundantly produced by the microenvironment of skeletal metastasis, the members of the transforming growth factor beta family (TGF-βs) are known to favor the metastatic process (5–7). The TGF-βs can amplify prostate cancer cell invasion capabilities in vivo and in vitro, stimulating tumor cell production of known biochemical modulators of tumor biology, such as parathyroid hormone-related peptide (PTHrP), a known survival factor for tumor cells and mediator of bone resorption, locally (3,5,7,10). Recently, the osteoclasts differentiation factor (RANK-ligand), a member of the tumor necrosis factor family (TNF family), documented to mediate the effects of PTHrP on osteoclast-mediated bone resorption stimulated by both breast and prostate cancer cells in experimental animals (15). Furthermore, other local bioregulators such as insulin-like growth factors (IGFs), basic fibroblast growth factors (bFGF), urokinase-type plasminogen activator (uPA)/uPA receptor bioregulation system, interleukins (ILs), endothelin-1, and bone morphogenetic proteins (BMP, particularly BMP-6) have shown to play a pivotal role in the osteoblastic metastasis from prostate cancer (3–11,16–20) (Fig. 1).

Recently, biochemical markers of bone resorption were increased both in the serum and urine of patients with advanced stage prostate cancer, suggesting that prominent bone formation in bone metastasis is actively coupled to an osteoclast-mediated bone resorption (3–6). Therefore, it appears that pharmacologic targeting of bone resorption can become an effective approach for a sizable number of patients with advanced prostate cancer (21). Furthermore, bone-derived growth factors, such as IGF-I, PTHrP, IL-6, bFGF, and TGF-β1 documented to exert survival factor activity on tumor cells, protecting prostate cancer cells from chemotherapy-induced apoptosis (9,10,22). These data generated the concept of anti-survival factor therapy (ASF therapy) in hormone refractory prostate cancer, which was designed to target the most important survival factor of bones, namely IGF-I (Fig. 2). This novel manipulation, a

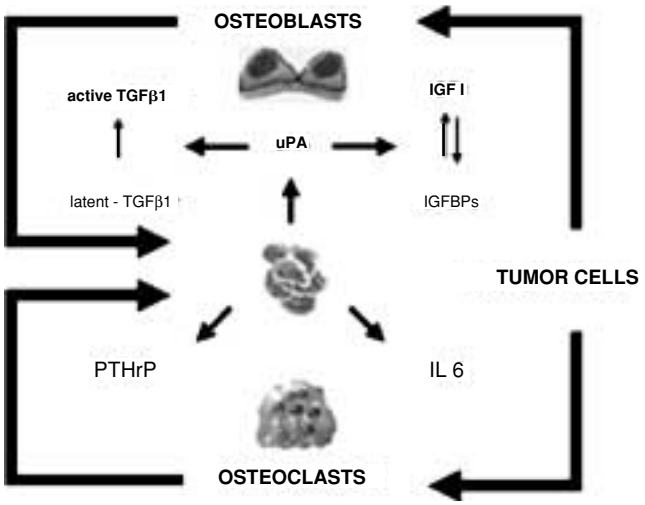

Fig. 1. Local mediators of tumor cell survival and tumor cell-induced osteoblastic reaction at the sites of skeletal metastasis in prostate cancer. Note the pivotal role of IL-6, PTHrP, and uPA/IGFBPs/IGFs/TGF-β1 bioregulation system into these processes.

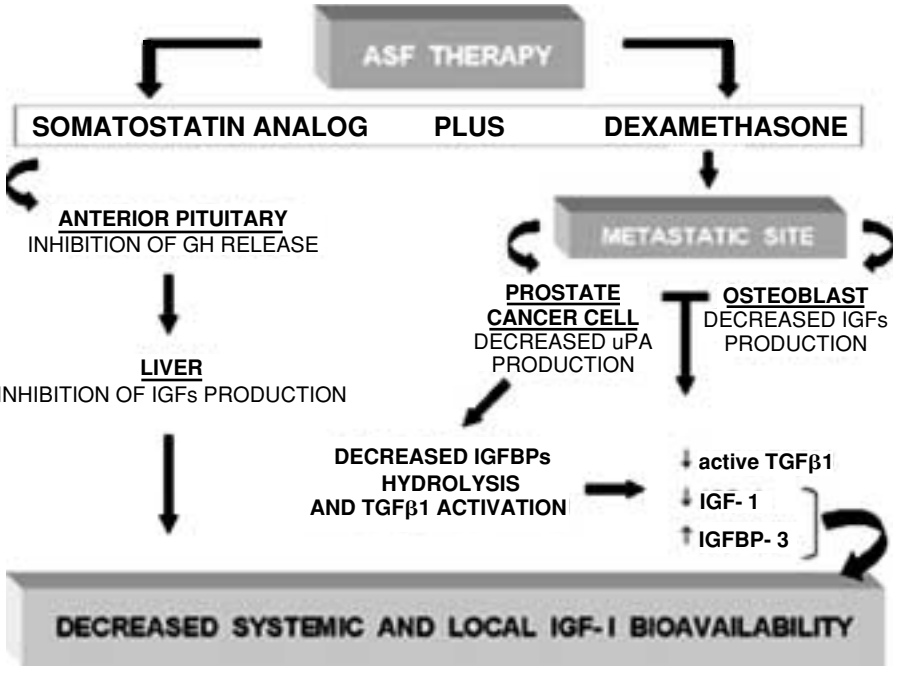

Fig. 2. Schematic representation of the main pharmacologic cell targets and pathophysiologic mechanisms targeted by ASF therapy (practically an anti–IGF-I bioavailability therapy) using the combination of somatostatin analog and dexamethasone in the clinical setting of hormone refractory prostate cancer.

paradigm of bone microenvironment-targeted therapy, produced objective and sustained clinical responses in androgen ablation of refractory prostate cancer (23,24). However, it is fair to conclude that today's available anticancer therapies cannot improve the median survival of metastatic prostate cancer patients. Consequently, efforts should focus on understanding the early events of the metastatic process in skeleton and developing molecular methods for detecting early tumor cell dissemination into peripheral blood and bone marrow (micrometastasis stage) (25–27).

The Use of Bisphosphonates in Prostate Cancer With Far Advanced Metastatic Disease in Bones

Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption, an action based predominantly on their ability to block the function of osteoclasts. Consequently, their use has been established in the management of medical disorders associated with an increased bone resorption, such as Paget's disease, multiple myeloma, osteolytic skeletal metastases in breast carcinoma, and the treatment of hypercalcemia associated with malignancy (21). Over the last decade they have been widely used in the management of osteoporosis, although at much lower dose regimens than those required in patients with overtly increased bone resorption. The exact mechanism by which bisphosphonates inhibit osteoclast activity has been recently clarified. Two major mechanisms have been described, depending on their chemical structure. Non-nitrogen-containing bisphosphonates, such as etidronate, clodronate, and tiludronate are intracellularly metabolized to substances toxic to the osteoclasts (28,29). Nitrogen-containing bisphosphonates, such as alendronate, ibandronate, olpadronate, pamidronate, and residronate, interfere with specific enzymes of the mevalonate pathway, eventually leading to a disruption of the cytoskeletal integrity and intracellular signaling of osteoclasts, resulting in their early apoptosis (30,31).

The rationale for the use of bisphosphonates in the management of metastatic prostate adenocarcinoma is not immediately obvious, given the predominant osteoblastic nature of these metastatic processes. The clinical use of the above agents in prostate cancer rests on a number of basic and clinical observations (32). There is ample evidence that prostate cancer metastases are also associated with increased bone resorption as determined by bone histomorphometry and biochemical markers of bone resorption (21,33–39). Notably in patients with metastatic prostate cancer, there is a progressive increase of urinary deoxypyridinoline excretion, which is associated with the increasing extent of bone metastases as evaluated by bone scan. In addition, there is a significant correlation between bone formation and bone resorption biochemical markers, suggesting the coupling between these two processes, although there is an apparent shift of the slope in favor of bone formation (35,36,40). Furthermore, the unusual presentation of hypercalcemia and its favorable response to bisphosphonate therapy also suggest, under certain circumstances, that the resorptive component of the skeletal disease may become predominant even in prostate cancer with osteoblastic metastasis. The latter can be either radiologically detected and/or histologically proven by the presence of an increased number of osteoclasts in metastatic sites (35–37).

Prostate cancer cells express bone-resorbing factors, such as macrophage colony-stimulating factor (MCSF), PTHrP, IL-1, and IL-6. These bone-resorbing factors may in turn be responsible for inducing the expression of RANK-ligand, and thereby inducing osteoclastogenesis (41–43). In this context, bisphosphonates can decrease the skeletal morbidity associated with bone metastases in breast carcinoma and multiple myeloma by reducing the incidence of pathologic fractures and episodes of hypercalcemia (44,45). In addition, there is in vitro evidence that bisphosphonates may inhibit the adhesion of prostate carcinoma cells to bone matrices or bone slices (46,47). Another possible mechanism of action of bisphosphonates in prostate cancer is their potential ability to inhibit matrix metalloproteinases enzymatic activity (48). Furthermore, both in vitro and in vivo data indicate that zoledronic acid, a third-generation bisphosphonate, has shown to exert, apart from its anti-osteoclastic and antitumor activities, anti-angiogenic effects, inhibiting the proliferation of human endothelial cells in vitro and angiogenesis induced by bFGF in tissue chamber implants in mice (49).

However, establishing an effect of bisphosphonates on growth and development of bone metastases is no simple task. As a part of a large phase III protocol, including more than 3000 patients with solid tumors and well-documented bony metastases, zoledronic acid (4 mg) was compared with placebo in 422 patients with hormone-refractory prostate cancer and bone metastases (50,51). Bone marker data from these trials confirmed that markers of bone resorption (N-telopeptide) as well as markers of bone formation (bone alkaline phosphatase) significantly reduced from baseline levels. More importantly, treatment with zoledronic acid resulted in a significant (25%) reduction in the proportion of patients with a skeletal-related event (SRE) and significantly reduced the skeletal morbidity rate (SMR) for all SREs compared with placebo. Furthermore, zoledronic acid significantly delayed the time to first SRE compared with placebo and had an impressive effect on bone pain in this trial (50,51).

Herein it is fair to outline that in past years several problems have marked the evaluation of bisphosphonates in metastatic prostate cancer because pain, the main complication of the bony metastases, is difficult to evaluate. Furthermore, most

studies with prostate cancer patients have generally included a small number of patients with far advanced disease, and the absorption of bisphosphonates has been difficult to establish, particularly with the use of oral preparations. A recent evidenced-based review of available studies on the potential use of bisphosphonates has understandably called for further investigations to establish the role of these agents in the palliative management of patients with metastatic prostate cancer (52). Apparently, the early promise of the first-generation bisphosphonate etidronate (53) has not been proven in a double-blind, placebo-controlled design (54). Recently, open-ended studies suggested that clodronate intravenously used and in adequate doses provided significant pain relief in patients with bony metastases (55–58). However, the only placebo-controlled study of this bisphosphonate published is surprisingly confounded by concomitant use of anticancer treatment (estramustine phosphate), so that the disease response and outcome is hard to interpret solely as bisphosphonate's efficiency and efficacy (59). The use of pamidronate, a nitrogen-containing bisphosphonate, was reported that is producing a significant but generally short-lived response, requiring intravenous administration at frequent intervals (60–66). Similarly, olpadronate was effective in the palliation of bone pain in 70% of patients with metastatic prostate cancer when it is given intravenously and followed by oral maintenance therapy; its clinical responses parallel biochemical changes in bone resorption (65).

In conclusion, bisphosphonate's efficiency is currently under investigation, however, deemed to be of limited clinical use in far advanced prostate cancer. Moreover, any conclusion regarding the ability of bisphosphonates to slow or prevent the metastatic process in prostate carcinoma must await results of large, controlled studies in which the agents are administered early in the course of the natural history of this malignancy. Apparently, effectiveness of bisphosphonates in preventing the evolution of bone metastasis can be made only in the clinical setting of localized prostate cancer patients at high risk for extraprostatic disease-bone involvement detected by molecular staging.

Understanding the Role of Bone Resorption at the Early Phase of Formation of Skeletal Lesions

The local mechanisms of initiating the establishment of metastasis in bones includes recognition and attachment of tumor cells onto bone marrow, invasion of bone matrix, and development of cell–cell interactions with bone cells, leading to blastic, lytic, or mixed-type lesions (2–7). This local process is initiated by the arrival of tumor cells, via peripheral blood, into the metabolically active ("red") bone marrow. Theoretically, at initial arrival in bone marrow tumor cells should be more or less equally distributed into the red bone marrow-containing bones (2–4). Obviously, a threshold number of circulating-invading tumor cells per milliliter of bone marrow is necessary for the establishment of clinically evident bony metastases (2). Based on extensive animal model data, a single prostate cancer metastatic foci would require more than 10,000 tumor cells circulating in the bloodstream (5,17). Consequently, bones of higher red bone marrow content are expected to be more frequently and more intensively invaded by tumor cells (4–7,67). Indeed, the predicted importance of red bone marrow content in the establishment of bony lesions is clinically confirmed by the frequency analysis of metastatic foci observed in bones rich versus bones poor in metabolically active bone marrow content (68,69).

At the stage of initial invasion of tumor cells into bone marrow (micrometastasis stage), tumor cells are recognized by bone marrow as foreign invaders and are forced to live in the hostile microenvironment of the immunologically hyperactive bone marrow cells. Consequently, tumor cell survival is directly linked to their ability to escape the intense immunologic surveillance of host tissue. This can be easily achieved by migration into bone matrix where bone matrix-residing cells (mainly osteocytes/osteoblasts) can protect and stimulate tumor cell proliferation by their production of growth/survival factors, such as IGFs, TGF-βs, BMPs, bFGF, and PTHrP (Fig. 1).

Indeed, the migration of tumor cells into bone matrix signals the transformation of micrometastasis stage, a diffuse dissemination of tumor cells into bone marrow to macrometastasis stage, which is mainly characterized by focal development of bony lesions (2–6,68). This mechanism, which enables the migration of tumor cells into bone marrow, involves the activation of osteoclast-mediated bone resorption locally. That includes tumor cell-mediated local attraction-fusion of circulating pre-osteoclasts to form mature osteoclasts, thereby initiating bone resorption and/or passive migration of tumor cells into bone matrix, capitalizing over the phenomenon of periodically activated bone resorption, a natural phenomenon that occurs approximately every 3–6 months at the bone remodeling units of red marrow-containing bones. There are no strong data to support the direct tumor cell resorption of bones in the absence of osteoclasts (Fig. 3). Therefore, osteoclast-mediated bone resorption is a crucial step for the formation of bone macrometastasis (3–7). Thereafter, late events taking place between tumor cells, already located into bone matrix and bone matrix-residing cells (osteoblast-osteocytes) will favor either the predominance of bone formation or the bone destruction, thereby resulting in the final histologic type of bony lesions (blastic, lytic or mixed) (Fig. 4).

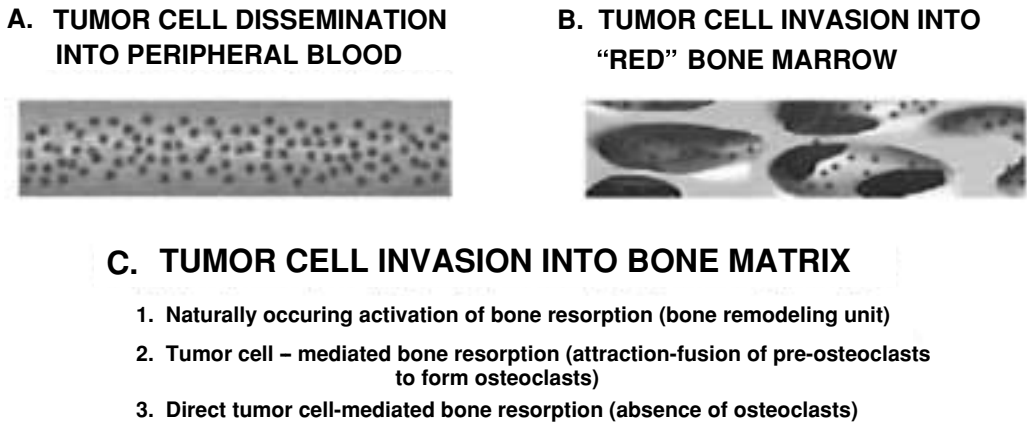

Fig. 3. Illustration of the initial steps implicated in the formation of bone metastasis. **(A)** Dissemination and survival of prostate cancer cells in the peripheral blood. **(B)** Dissemination of tumor cells into the metabolically active red bone marrow (micrometastasis stage). **(C)** Activation of bone resorption, which enables the migration of tumor cells into bone matrix and the establishment of macrometastasis in bones.

Stratification of Risk for Bone Involvement in Patients With Clinically Localized Prostate Cancer

Apparently, our inability to treat advanced prostate cancer is mainly caused by the limitations of our diagnostic tools to detect bony lesions at early phase of tumor cell dissemination into bone marrow, the so-called micrometastasis stage. It is conceivable that development of molecular tools enabling the detection of micrometastasis stage could provide the opportunity for an effective therapeutic intervention right before tumor cell migration into bone matrix (macrometastasis stage).

Indeed, the enhanced sensitivity nested RT-PCR detection of prostate-specific antigen (PSA) and/or prostate specific membrane antigen (PSMA) mRNA is capable of detecting the presence of 1 prostate cell in 10^6 noncancer peripheral blood nucleated cells and bone marrow aspirates (70–75). Initially, this enhanced sensitivity RT-PCR assay was thought to be an excellent approach for evaluating clinical samples due to PSA and PSMA specificity to prostate cells. However, PSA mRNA expression was documented in non-prostate origin cell lines and enhanced sensitivity PCR assays revealed the presence of illegitimate PSMA transcripts in peripheral blood leukocytes and other non-prostate cancer cell lines (76,77). Therefore, any further attempt to obtain enhanced analytical sensitivity PCR assays was bound to suffer a decrease in the assay's specificity, thereby compromising the clinical usefulness of molecular staging in patients with prostate cancer (75).

Recently, the clinical relevance of molecular staging was reassessed by modifying the definition of a positive and negative RT-PCR detection, using certain clinically established prerequisites (78–80). Based on these prerequisites, positive molecular staging status is awarded only to samples tested positive for both PSA and PSMA transcripts. Consequently, samples found, in a repetitive manner, to be of a differential RT-PCR status for tumor markers detection (PSA positive/PSMA negative or vice versa) were classified as negative and differential RT-PCR detection was attributed to illegitimate transcription (78,79). In addition, the sensitivity of the RT-PCR assays was set at 3–5 LNCaP prostate cancer cells in 10^6 peripheral blood nucleated cells for the screening of patients samples, which is considered to reflect tumor cell concentration, a more relevant criteria for the establishment of metastasis in animal models (78). These specificity/sensitivity

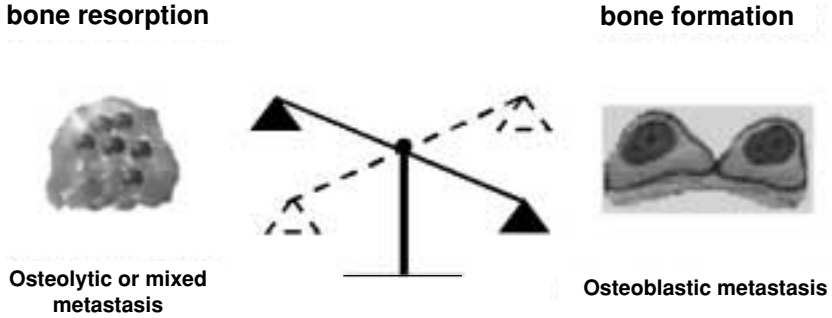

Fig. 4. Schematic illustration of the histologic types of bony lesions, a result of specific cell–cell interactions established at the sites of metastasis. Tumor cell interactions with osteoblasts and osteoclasts can be coupled in a manner that can favor either bone formation or bone resorption, resulting in the development of osteoblastic, osteolytic, and/or mixed histologic types of bony lesions.

RT-PCR conditions were tested on total RNA extracts obtained from blood donor clinics (blood samples of young men and women) expected anyway to be negative for PSA and PSMA transcripts, RNA extracts of samples from patients with biopsy-proven benign prostatic hyperplasia, and RNA samples of patients with far advanced disease in bones (78,79).

Indeed, data of such comprehensive molecular staging procedure performed in peripheral blood (PB) and bone marrow biopsy (BM) showed strong correlation of positive PSA and PSMA RT-PCR detections with *(1)* PSA levels ≥20 ng/ml at diagnosis, *(2)* Gleason's scores ≥7 at diagnosis, and *(3)* time-to-biochemical failure shortly after radical prostatectomy in patients with clinically localized prostate cancer. Under these experimental conditions, molecular staging enabled the stratification of patients into groups with *(1)* low risk for bone involvement-negative analysis for both PSA and PSMA in PB and BM, *(2)* high risk for bone involvement-positive analysis for both PSA and PSMA in PB and BM, and *(3)* intermediate risk for extraprostatic involvement-positive analysis for both PSA and PSMA at PB but negative for both PSA and PSMA in BM (74,75). We recently analyzed the results of molecular staging in 111 patients with clinically localized prostate cancer (80). These data confirm the previously reported association of positive molecular staging with higher PSA and Gleason's score values at diagnosis as compared with those of negative molecular staging. Kaplan-Meier analysis and log-rank tests reveal that the median time-to-PSA failure (biochemical failure) was significantly different between groups with positive and negative molecular staging either in PB or BM. During the follow up, 20% of the patients with positive molecular staging have become positive for bony metastasis, as detected by bone scan and confirmed by computerized tomography (CT). These data suggest that positive molecular staging in PB and BM can define the patients of high risk for disease progression/extraprostatic growth (80).

Consequently, comprehensive molecular staging procedure is able to stratify patients with clinically localized disease into groups of high risk and low risk for extraprostatic disease and bone involvement (Fig. 5).

Conclusion

Because, osteoclast-mediated bone resorption has a permissive role for the establishment of bony

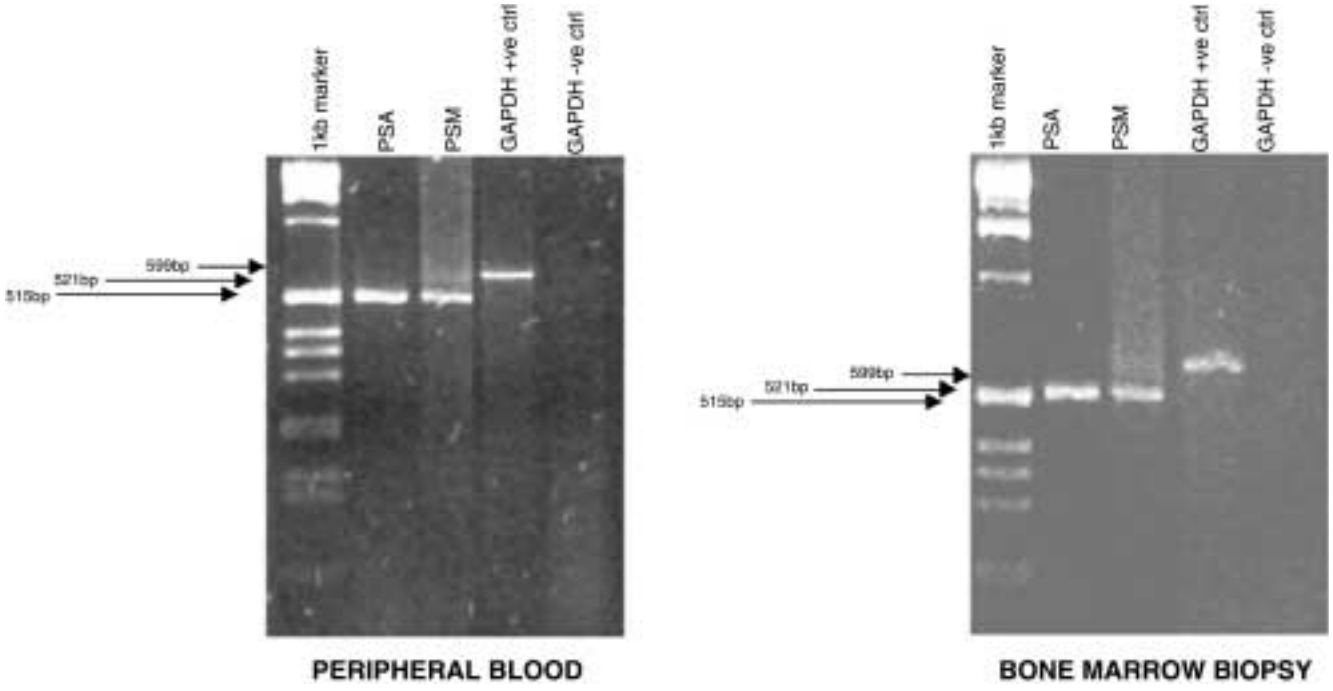

Fig. 5. An example of comprehensive molecular staging in a clinically localized prostate cancer patient using RT-PCR detection of PSA and PSMA transcripts in PB and BM as developed by the clinical prerequisites previously described (74,75). This example represents the case of patient with PSA = 5.6 ng/ml; Gleason's score = 7 (4 + 3) prostatic adenocarcinoma (transrectal ultrasound-guided biopsy); negative evaluation for metastasis as assessed by bone scan, metastatic survey (x-rays) and CT; who tested positive for both PSA and PSMA in PB and BM. He underwent immediate radical prostatectomy (RP) and experienced early biochemical failure (PSA = 1.5 ng/ml) 6 months after RP. Then he was prescribed local irradiation therapy plus temporary combined androgen blockade (CAB; for 3 months). Twelve months after irradiation therapy, PSA was 9.0 ng/ml, and the bone scan revealed the presence of multiple sites of bone lesions throughout the spine and pelvis. The latter were confirmed by CT. Notably, RT-PCR–based indications for probable extraprostatic disease and bone involvement were given approximately 2 years before the final diagnosis of stage D2 disease.

macrometastasis and comprehensive molecular staging can detect prostate cancer patients at high risk for bone involvement, it is reasonable to postulate that bisphosphonate-based blockade of osteoclast-mediated bone resorption at this initial stage of the disease dissemination into red bone marrow can inhibit the formation of macrometastasis by inhibiting the migration of tumor cells into bone matrix (79,80).

Acknowledgments

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