Journal of Cellular Biochemistry 96:439–446 (2005)

PROSPECTS

A Paradigm for the Treatment of Prostate Cancer Bone Metastases Based on an Understanding of Tumor Cell–Microenvironment Interactions

Robert D. Loberg,* Bishoy A. Gayed, Karin B. Olson, and Kenneth J. Pienta

Departments of Urology and Internal Medicine, University of Michigan Urology Center, The University of Michigan, Ann Arbor, Michigan

Abstract The pliability of cancer cells to mutate into several different phenotypes in an attempt to find one that will survive and colonize at the metastatic site is a tremendous ''hurdle'' to overcome in designing novel cancer therapeutics. New targets of therapy are essential if we are to effectively overcome the evasiveness of cancer. The interaction between the tumor cell and the surrounding microenvironment creates a vicious cycle that perpetuates disease survival and progression. The future of cancer therapy resides in the ability to focus on the recruited and exploited relationships of the cancer cell with the host environment. These therapies target cancer cell growth early and interrupt the vicious cycle that is created by the tumor cells interacting with bone components by inhibiting osteoclasts, osteoblasts, stromal cells, and endothelial cells. They alter the bone microenvironment, creating a hostile ''soil'' that prevents the ''seed'' from developing into bone metastases and represent a potential new platform for the development of prostate cancer therapeutics. J. Cell. Biochem. 96: 439–446, 2005.

Key words: prostate cancer; bone metastasis; cancer therapy

In recent years, the incidence of prostate cancer has risen consistently and the disease is responsible for more gender-specific cancer-related deaths in men than any other cancer. The American Cancer Society estimates that during 2005 about 232,090 new cases of prostate cancer will be diagnosed in the US and 30,350 men will die of metastatic disease. About 1 man in 5 will be diagnosed with prostate cancer during his lifetime, and 1 man in 33 will die of this disease. As the population ages, these numbers are expected to increase. Prostate cancer is curable if detected when confined to the prostate gland but attempts at treatment and cure have met with limited success once the disease has spread outside the prostate.

Approximately 80% of patients who have died of advanced hormone refractory prostate cancer have clinical evidence of bone metastases and 100% have histologic bone involvement [Saitoh et al., 1984; Bubendorf et al., 2000; Roudier et al., 2003]. The development of bone disease is believed to follow the seed and soil hypothesis first described by Stephen Paget in 1889 [Fidler, 2003]. In advanced prostate cancer patients, tumor cells (seed) in the bloodstream invade the marrow space (soil) forming osseous lesions. Metastatic prostate cancer bone lesions are the result of a complex interplay between the cancer cells themselves and the bone microenvironment, resulting in a heterogeneous disease that induces a combination of both osteolytic and osteoblastic lesions. The pathogenesis of osteolytic and osteoblastic bone metastases involves interactions between osteoclasts, osteoblasts, endothelial cells, fibroblasts, adipocytes, bone marrow precursor cells, cells of the immune system, the extracellular matrix (ECM), and the cancer cells (Fig. 1). A paradigm shift from an initial treatment strategy that primarily targets the tumor cell directly, i.e., traditional

Grant sponsor: NIH 1; Grant numbers: R01 CA102872, PO1 CA093900-01A2; Grant sponsor: The Prostate Cancer Foundation.

*Correspondence to: Robert D. Loberg, PhD, University of Michigan Urology Center, 7431 CCGC, 1500 E. Medical Center Dr., Ann Arbor, MI 48109-0946.
E-mail: rloberg@umich.edu

Received 5 April 2005; Accepted 6 April 2005

DOI 10.1002/jcb.20522

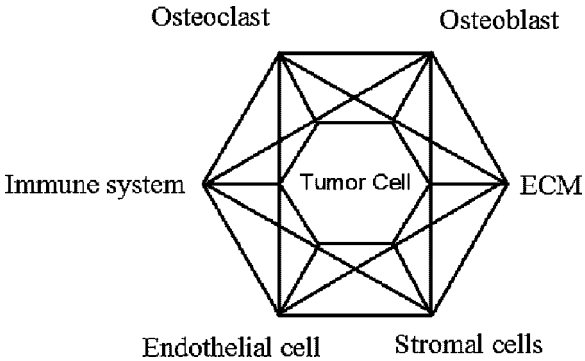

Fig. 1. The hexagon of prostate tumor cell–bone microenvironment interactions. The relationship between the tumor cell and the surrounding tissue is a complex environment. Tumor cells interact with the extracellular matrix (ECM), stromal cells, immune system, osteoblasts, osteoclasts, and endothelial cells to coordinate a sophisticated series of interactions to promote tumor cell survival and proliferation leading to a ''hexagon of pain'' for patients with advanced prostate cancer. These interrelationships identify a paradigm shift in understanding prostate cancer growth in bone and lead to the ability to design targeted therapies to interrupt the vicious cycle of the tumor cell with its microenvironment.

chemotherapy, to new therapies that exploit the interactions and contributions of the various cells and elements of surrounding microenvironment to the development of the metastatic lesions is now being explored and already exploited to improve outcomes for patients with advanced prostate cancer.

DEVELOPMENT OF A NEW PARADIGM FOR THE TREATMENT OF PROSTATE CANCER COMBINING TARGETED THERAPIES

Targeting the Tumor Cell Burden Early in the Metastatic Process

The canonical treatment paradigm for virtually all cancers, including prostate cancer, has been to target the tumor cell directly. Chemotherapy is generally used in patients with advanced, clinically evident disease. Recently, for the first time, trials have demonstrated that treatment with docetaxel increases survival in patients with hormone refractory prostate cancer [Tannock et al., 2004]. Current chemotherapy regimens may debulk the tumor and palliate symptoms, but cannot completely eradicate late stage disease. The advent of prostate specific antigen (PSA) as a molecular marker has made early detection of disease and disease recurrence possible. Disease recurrence after radical prostatectomy can now be reliably detected in patients with a PSA of 0.1 ng/ml or potentially lower with supersensitive assays [Yu et al., 1997]. In addition, circulating tumor cells can be detected in many of these patients with rising PSA, suggesting that the cancer is capable of spreading early in the disease course [Loberg et al., 2004]. It is generally considered that a serum PSA of 4.0 ng/ml is roughly equivalent to a tumor of one cubic centimeter which is equivalent to one billion tumor cells [Fukatsu et al., 2003]. It is possible, therefore, to potentially detect tumor recurrence when there are approximately 1–25 million cells present.

The argument for treating micrometastatic disease early in the cancer course is not novel and is based on at least four premises [Norton and Simon, 1977; DeVita, 1983]. First, in a perfect system, in vitro or in vivo, one dose of chemotherapy kills one log of tumor cells [DeVita, 1983]. By simple tumor burden, it is easier to eradicate a smaller tumor with the same number of chemotherapy cycles compared to a large tumor. Second, microscopic foci of cancer cells grow at an exponential rate and are easier to eradicate with traditional cytotoxic agents [Norton and Simon, 1977]. Third, as a tumor grows, heterogeneity of tumor cells continues to evolve with resulting chemo-resistance. Fourth, prostate cancer cells secrete several factors that stimulate the surrounding bone microenvironment which in turn promotes growth of the cancer cells, creating a vicious cycle of tumor cell–microenvironment interactions that drive the progression of metastatic disease [Chung, 2003]. The cancer cells secrete factors which stimulate osteoclasts to break down bone, as well as proteases such as PSA and urokinase which break down the ECM. This catabolism of the bone microenvironment results in the release of multiple factors that activate osteoblasts and stimulate the growth of the cancer cells (see Fig. 2).

Targeting Osteoclasts and Osteolysis

Although osseous metastatic lesions from prostate cancer are predominantly osteoblastic, the presence of osteoclastic activity within metastatic lesions is also vitally important to the understanding and treatment of prostate cancer. Osteoclasts are a unique cell type derived from hematopoetic stem cells and express a large number of highly specific enzymes designed to breakdown bone. These

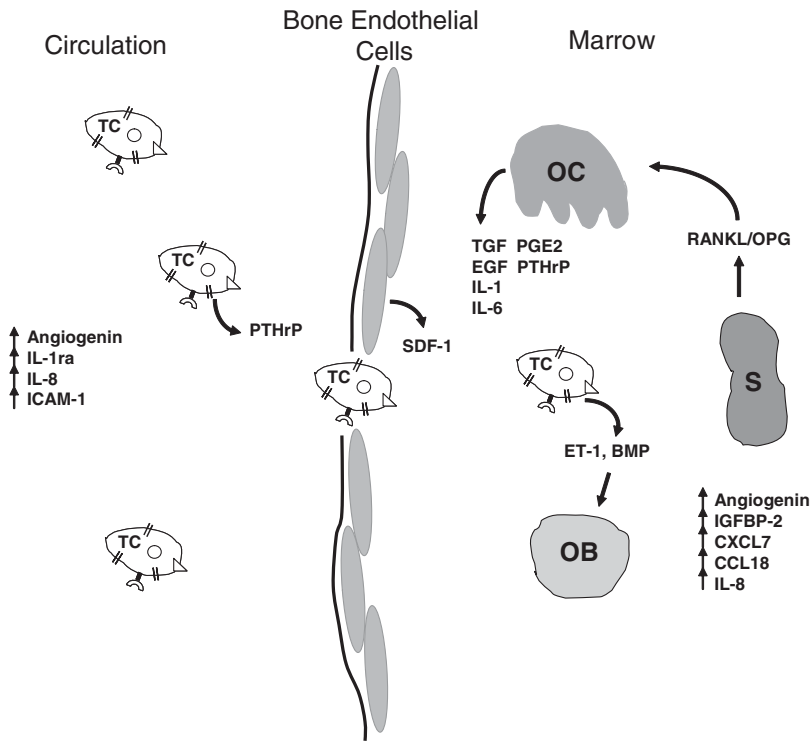

Fig. 2. Diagrammatic representation of bone-specific prostate cancer metastasis. A variety of factors are released from both the tumor cell and the bone microenvironment that participate in successful metastasis and growth of neoplasms. [TC, tumor cell; OB, oseteoblast; OC, osteoclast; S, stromal cell; PTHrp, parathyroid hormone related protein; SDF, stromal derived factor; IL-1, interleukin 1; IL-1ra, interleukin 1 receptor antagonist; IGFBP-2, insulin-like growth factor binding protein 2; IL-6, interleukin 6; IL-8, interleukin 8; PGE2, prostaglandin E-2; TGF, transforming growth factor; EGF, epidermal growth factor; BMP, bone morphogenic protein; ET-1, endothelin-1; CXCL7, chemokine (C-X-C motif) ligand 7; CCL18, chemokine (C-C motif) ligand 8; ICAM-1, intracellular adhesion molecule-1].

cells are known to be activated by a variety of growth factors, many of which are secreted by prostate cancer cells (i.e., TGFβ, EGF, IL-1, IL-6 etc.). The activation of osteoclasts leads to the catalysis of the bone matrix and subsequent release of multiple growth factors and cytokines which are capable of stimulating metastatic growth in the bone [Keller, 2002]. The release of these factors aid in further chemotaxis of prostate cancer cells and also creates an environment suitable for prostate cancer growth and proliferation.

Several strategies have been developed to inhibit osteoclast activity. Newer, nitrogen-containing IV bisphosphonates, which target osteoclasts and osteolytic lesions, have recently proven efficacious in the palliative care of prostate cancer patients. Bisphosphonates are analogs of pyrophosphate, a normal constituent of the bone matrix, which binds to bone surfaces (hydroxyapatite crystals) making them less available to osteoclast resorption. Additionally, bisphosphonates inhibit recruitment of osteoclast precursors, prevent the migration of osteoclasts towards bone, and inhibit the production of prostaglandin-E2, Interleukin-1, and other proteolytic enzymes. Treatment of hormone refractory prostate cancer patients with chemotherapy plus the bisphosphonate zolendronic acid (Zometa®) demonstrated a decrease in skeletal related events such as fractures and the need for palliative radiation therapy. Zolendronic acid is now approved as an adjunctive treatment for patients with androgen independent prostate cancer [Winquist and Berry, 2004].

Other strategies to inhibit osteoclast activity are under development, including inhibition of the parathyroid related peptide (PTHRP) and RANK-L/RANK signaling axes which induce osteoclast activity [14]. Selective estrogen receptor modulators (SERMs) may be able to decrease the bone resorption associated with bone metastases. On a systemic level, SERMs

affect parathyroid hormone activity (PTH) and Vitamin D metabolism resulting in altered calcium homeostasis [Jiann et al., 2002]. The local effects of SERM therapy include the inhibition of osteoclast activity, the inhibition of osteoblast activity, and direct cytotoxic effects to tumor cells [Taranta et al., 2002]. Thus, SERMs (e.g., tamoxifen, clomiphene, and raloxifene) are non-steroidal compounds that act as estrogen receptor agonists and antagonists depending on the target tissue.

Treatment with osteoclast inhibitors early in disease recurrence may delay the development of osseous metastases by altering normal bone homeostasis. In animal models it has been demonstrated that prostate cancer cells "seed" or "home" to areas of high bone metabolism [Kalikin et al., 2003]. It can be postulated, therefore, that decreasing bone metabolism via long-term osteoblastic inhibition throughout the disease course may decrease successful seeding of tumor cells in potential metastatic sites. At least one clinical trial demonstrated an increase in survival with long-term bisphosphonates treatment in women with metastatic breast cancer [Diel et al., 2004]. Clinical trials testing this concept in early recurrent prostate cancer are underway.

Targeting Osteoblast and Bone Formation

The most common histology of prostate cancer bone metastases is a disorganized osteoblastic response and the osteoblast plays a key role in the bone metastasis microenvironment. Several growth factors (e.g., insulin-like growth factor 1, transforming growth factor beta, endothelin-1, fractalkine) produced by osteoblasts and osseous stromal cells act as chemoattractants for prostate cancer cells, and also promote tumor growth and proliferation [Shulby et al., 2004].

To date, targeting the osteoblasts has been an underdeveloped area of research, however, the compounds that inhibit the endothelin axis have proven to be an excellent example of osteoblast targeted therapy. Endothelin-1 (ET-1) was originally identified as a vasoactive peptide released from the vascular endothelium to regulate blood pressure and vascular tone by promoting vasoconstriction [Withrington et al., 1989]. Recently, ET-1 has been shown to be an important mediator of tumor growth and tumor cell survival in prostate cancer [Mohammad and Guise, 2003]. Initial evidence in both breast cancer and prostate cancer preclinical models demonstrated that the use of specific receptor antagonists to ET-1 could reduce the rate of bone formation and the frequency of bone metastases both in vitro and in vivo [Mundy, 2002; Guise and Mohammad, 2004]. Several compounds are under investigation as a novel class of chemotherapeutic agents that target the ETA (ET-1 receptor), including Atrasentan, an orally bioavailable ETA receptor inhibitor, disrupts the bone microenvironment by inhibiting the osteoblastic axis and has been studied in several phase II clinical trials in prostate cancer [Lee, 2003; Nelson et al., 2003; Zonnenberg et al., 2003; Nelson, 2005].

Targeting the Stromal Cells

The stromal elements that participate in and support cancer growth include fibroblasts, smooth muscle cells, endothelial cells, and inflammatory cells [11]. It appears that targeting these stromal components of the microenvironment may be effective in reducing the development of metastases. Bagshaw and colleagues noted that there was a reduction in the frequency of metastatic lesions to the lumbar spine in prostate cancer patients treated prophylactically with high dose radiation (35–60 Gy) to the lumbar spine [Bagshaw et al., 1990]. A similar finding was reported in breast cancer patients treated with sternal radiation [Grimard et al., 1988]. While radiation can potentially eradicate micrometastatic disease, it has also been postulated that these doses of radiation are more than adequate to destroy the replicative potential of the bone endothelial cells. This would result in the inability to vascularize a growing tumor in these radiated sites [Abdollahi et al., 2003]. Irradiation may also reduce bone metastases by inducing other bone stromal cell damage, osteocyte damage, and/or osteoblast damage, creating an unfavorable microenvironment for the tumor cells to colonize.

Another approach to altering the microenvironment utilizes systemic radioisotopes instead of local irradiation. Radioisotopes exert their palliative effect by combining with the calcium component of hydroxyapatite in damaged bone [Neves et al., 2002]. Radioisotopes are effective therapeutic agents for the management and palliation of bone-specific disease due to the high levels of retention by bone metastases (highest in damaged bone, less in normal bone

tissue, and no retention outside of bone). The most common radioisotopes are strontium-89 and samarium-135 lexidronam ethylene diamine tetramethylene phosphonate [Graham et al., 1999; Dafermou et al., 2001; Palmedo et al., 2003]. These radionuclides are beta-emitting radioisotopes that differ primarily in their radioactive half-lives and the tissue penetrance of their beta particles. Adding systemic radioisotopes to chemotherapy regimens for patients with advanced prostate cancer appears to increase response rates and survival [Tu et al., 2001; Akerley et al., 2002]. Further exploration of how this could be applied to the human disease setting is being investigated in preclinical and clinical settings.

Targeting Cytokine and Growth Factor Stimulation of Endothelial Cells

Another important pathway for targeting the tumorigenic potential of the microenvironment is through the interruption of cytokine and growth factor production. Multiple cytokines are important in cancer development, both those that stimulate cancer cell growth directly and those that target the growth of supporting cells such as endothelial cells. Vascular endothelial growth factor (VEGF) has emerged as the primary cytokine secreted by tumor cells which stimulates new blood vessel growth. The VEGF family of growth factors consists of five related proteins which have been implicated in angiogenesis (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E). There are three VEGF receptors (VEGFR1, VEGFR2, and VEGFR3) that appear to play an important role in the angiogenesis of prostate cancer [Drevs et al., 2005]. One strategy to utilize this pathway in prostate cancer treatment is to block the interaction of VEGF with its receptors. This can be done by using antibodies to VEGF (i.e., bevacizumab), antibodies to the VEGF receptors themselves, or by inhibiting the tyrosine kinase activity of the receptor. One example of this type of agent being tested in androgen independent prostate cancer phase II trials is PTK787, an orally administered amino-phthalazine that blocks all known VEGF receptors [Drevs et al., 2005].

A second strategy targeting the microenvironment involves inhibiting the growth of new blood vessels by blocking integrin function. Tumor-related blood vessels sprout into the ECM in a process that is mediated by the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ which bind to a variety of ECM molecules including vitronectin [Maragoudakis et al., 2002]. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ bind factors such as vitronectin containing the amino acid sequence Arg-Gly-Asp (RGD), which appears to be critical in mediating the ligation signal, allowing endothelial cells to attach to the ECM [Meerovitch et al., 2003]. This interaction can be blocked by EMD 121974 (Cilengitide), the inner salt of a cyclized pentapeptide, or an antibody to $\alpha_v\beta_3$ (Vitaxin) [Posey et al., 2001]. Currently, both compounds are in clinical trials for androgen independent prostate cancer. It is generally believed that targeting tumor-associated blood vessel growth is a more successful therapeutic strategy when a tumor is small, and should be applied early in the disease course.

Targeting the Destruction of the Extracellular Matrix

The ECM of the bone includes not only the calcium hydroxyapatite of the bone itself but also the traditional ECM of the stromal and cancer cells. As a tumor grows, it releases multiple proteases including PSA, urokinase, and matrix metalloproteinases which break down the ECM to allow for growth, invasion of new blood vessels, and metastasis. Several therapy modalities have been tried and are currently under investigation that targets these enzymes. Tetracyclines are a family of compounds (e.g., doxycycline and tetracycline) that have been used as antibiotics but have recently been shown to inhibit matrix metalloproteinases (MMPs). MMPs are known to be essential to the invasion and metastatic potential of many cancers [Saikali and Singh, 2003]. Currently Col-3, a modified tetracycline molecule, is in phase I clinical trials in a variety of solid tumors including hormone refractory metastatic cancer [Rudek et al., 2001]. The effects of tetracycline and doxycycline on MMP activity has led to the development of ongoing clinical trials in breast cancer and prostate cancer [Saikali and Singh, 2003]. Green tea may also inhibit MMPs activity. Green tea has been shown to have important chemopreventive properties attributed to its high polyphenolic content, specifically epigallocatechin-3-gallate (EGCG) [Adhami et al., 2003]. Administration of green tea to the transgenic adenocarcinoma of the mouse prostate (TRAMP) model resulted in an inhibition of VEGF and MMP activity [Adhami et al., 2003].

Targeting the Immune System With Vaccines

Cancer cells evade the immune system through multiple strategies and a considerable amount of research has focused on targeting the immune system either by enhancing the immunogenicity of the tumor cells directly or inducing a more effective immune response. APC8015 (Provenge®) is an investigational therapeutic vaccine that uses autologous antigen presenting cells containing a recombinant fusion protein of prostatic acid phosphatase linked to a molecule that specifically targets a receptor expressed on the surface of human prostate cancer cells [Burch et al., 2004; Schellhammer and Hershberg, 2005]. Initial results have demonstrated activity in androgen independent prostate cancer and have encouraged further clinical investigations. A randomized phase III trial studying the effectiveness of APC8015 in attenuating disease progression and disease-related pain in patients who have asymptomatic hormone refractory prostate cancer is currently is accrual [http://www.cancer.gov/clinicaltrials].

An alternative approach to targeting the immune system is to target the complement system. For example, cancer cells overexpress CD55, a membrane-bound complement regulatory protein that is overexpressed in a variety of cancers and protects cells against complement-mediated lysis. Currently, an anti-idiotypic monoclonal antibody, 105AD7, is in clinical trials as a cancer vaccine that mimics the tumor-associated antigen CD55 and has demonstrated the applicability of vaccine-targeted approaches in cancer treatment. These strategies could be used in conjunction with chemotherapy and as primary therapy early in the disease course.

CONCLUSION/PERSPECTIVE

Table I demonstrates a provocative approach used to treat a patient with early recurrent prostate cancer utilizing many of the approaches discussed previously. J.P, a young patient who failed radical prostatectomy, salvage radiation therapy, and hormonal therapy who now has biochemical recurrence without additional evidence of disease. He received three 28 day cycles of multi-targeted therapy including docetaxel 30 mg/m^2 on days 2 and 9, carboplatin on day 2 to an AUC of 5.0, bevacizumab 5 mg/kg on day 9, cyclophosphamide 100 mg daily for 20 days of every 30 days, thalidomide 100 mg daily, calcitriol 0.5 mcg/kg on days 1 and 8, estramustine 140 mg three times a day and etoposide 50 mg two times a day on days 1, 2, 3 and 8, 9, 10, prednisone 10 mg daily and GM-CSF 250 mcg three times per week. He received 4 mg zolendronic acid on day 2. He then received doxorubicin 10 mg weekly $\times 6$ and one dose of strontium-89 during the second week of doxyrubicin. This multi-modality therapy targeted early cancer cell growth through combined chemotherapy and in addition, attempted to interrupt the vicious cycle created by interactions between the tumor and key components of the bone microenvironment.

Cancer should be considered a multi-cellular organ involving both heterogenous cancer cells

TABLE I. One Example of a Hypothetical Treatment Strategy for a Patient With Early Recurrent Prostate Cancer

Treatment	Rationale
Chemotherapy + hormonal therapy[a]	Hormonal and chemotherapy will kill cancer cells when they exist in fewer numbers. Also decreases release of proteases that destroy bone ECM
Bisphosphonate or selective estrogen receptor modulator treatment	Inhibit bone destruction by osteoclasts. Also inhibits release of tumor growth factors from the bone matrix
Endothelin 1 inhibitor administration[b]	Inhibit osteoblast activity. Inhibits osteoblast stimulation of osteoclasts through the RANK-RNKL axis as well as the release of tumor stimulating cytokines
Limited radiotherapy to lumbar spine[c]	100% of patients who die with prostate cancer have metastases to their lumbar spine, suggesting that this is a first stop metastatic spread of prostate cancer and the most likely place that early disseminated disease is harbored
Treatment with radioisotope[d]	Radioisotopes will target early cancer lesions where destruction of bone is occurring secondary to the presence of cancer
Administration of a angiogenesis inhibitor[b]	Tumors cannot grow without neovascularization. Most data suggests that angiogenesis inhibitors work better in earlier disease settings
Administration of tetracycline or green tea[b]	Inhibition of proteases

[a]Limited treatment with chemotherapy (six cycles) and 2–3 years of hormonal therapy bases on tumor kinetics and data from adjuvant radiation studies (9, 49).
[b]Chronic oral treatment.
[c]Treatment with 30–45 Gy (30).
[d]Treatment one time.

as well as multiple normal cell types interacting with the tumor cells. Targeting the tumor cell itself represents only one avenue for attacking the disease of advanced prostate cancer. In order to develop effective therapeutic strategies it is imperative that we move beyond the concept of cancer as "tumor cell" and expand the therapeutic target options to include the cancer as "organ." A promising avenue of cancer therapy resides in the ability to focus on the recruited and exploited relationships of the cancer cell with the host environment.

ACKNOWLEDGMENTS

Supported by 2 P50 CA69568-06A1. Dr. Pienta is supported by the American Cancer Society as a Clinical Research Professor.

REFERENCES

Abdollahi A, Lipson KE, Han X, Krempien R, Trinh T, Weber KJ, Hahnfeldt P, Hlatky L, Debus J, Howlett AR, Huber PE. 2003. SU5416 and SU6668 attenuate the angiogenic effects of radiation-induced tumor cell growth factor production and amplify the direct anti-endothelial action of radiation in vitro. Cancer Res 63:3755–3763.

Adhami VM, Ahmad N, Mukhtar H. 2003. Molecular targets for green tea in prostate cancer prevention. J Nutr 133:2417S–2424S.

Akerley W, Butera J, Wehbe T, Noto R, Stein B, Safran H, Cummings F, Sambandam S, Maynard J, DiRienzo G, Leone L. 2002. A multiinstitutional, concurrent chemoradiation trial of strontium-89, estramustine, and vinblastine for hormone refractory prostate carcinoma involving bone. Cancer 94:1654–1660.

Bagshaw MA, Cox RS, Ramback JE. 1990. Radiation therapy for localized prostate cancer. Justification by long-term follow-up. Urol Clin North Am 17:787–802.

Boyle WJ, Simonet WS, Lacey DL. 2003. Osteoclast differentiation and activation. Nature 423:337–342.

Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC, Mihatsch MJ. 2000. Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. Hum Pathol 31:578–583.

Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, Richardson RL, Valone FH, Vuk-Pavlovic S. 2004. Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: A Phase 2 trial. Prostate 60:197–204.

Chung LW. 2003. Prostate carcinoma bone–stroma interaction and its biologic and therapeutic implications. Cancer 97:772–778.

Dafermou A, Colamussi P, Giganti M, Cittanti C, Bestagno M, Piffanelli A. 2001. A multicentre observational study of radionuclide therapy in patients with painful bone metastases of prostate cancer. Eur J Nucl Med 28:788–798.

DeVita VT Jr. 1983. The James Ewing lecture. The relationship between tumor mass and resistance to chemotherapy. Implications for surgical adjuvant treatment of cancer. Cancer 51:1209–1220.

Diel IJ, Body JJ, Lichinitser MR, Kreuser ED, Dornoff W, Gorbunova VA, Budde M, Bergstrom B, MF 4265 Study Group. 2004. Improved quality of life after long-term treatment with the bisphosphonate ibandronate in patients with metastatic bone disease due to breast cancer. Eur J Cancer 40:1704–1712.

Drevs J, Zirrgiebel U, Schmidt-Gersbach CI, Mross K, Medinger M, Lee L, Pinheiro J, Wood J, Thomas AL, Unger C, Henry A. 2005. Soluble markers for the assessment of biological activity with PTK787/ZK 222584 (PTK/ZK), a vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor in patients with advanced colorectal cancer from two phase I trials. Ann Oncol 16:558–565.

Fidler IJ. 2003. The pathogenesis of cancer metastasis: The 'seed and soil' hypothesis revisited. Nat Rev Cancer 3:453–458.

Fukatsu A, Ono Y, Ito M, Yoshino Y, Hattori R, Gotoh M, Ohshima S. 2003. Relationship between serum prostate-specific antigen and calculated epithelial volume. Urology 61:370–374.

Graham MC, Scher HI, Liu GB, Yeh SD, Curley T, Daghighian F, Goldsmith SJ, Larson SM. 1999. Rhenium-186-labeled hydroxyethylidene diphosphonate dosimetry and dosing guidelines for the palliation of skeletal metastases from androgen-independent prostate cancer. Clin Cancer Res 5:1307–1318.

Grimard L, Eapen L, McDermot R, Genest P, Nair R. 1988. Does parasternal irradiation reduce thoracic vertebral metastases in breast cancer? Int J Radiat Oncol Biol Phys 14:565–569.

Guise TA, Mohammad KS. 2004. Endothelins in bone cancer metastases. Cancer Treat Res 118:197–212.

Jiann BP, Lu YC, Chang HT, Huang JK, Jan CR. 2002. Effect of clomiphene on $Ca(2^{+})$ movement in human prostate cancer cells. Life Sci 70:3167–3178.

Kalikin LM, Schneider A, Thakur MA, Fridman Y, Griffin LB, Dunn RL, Rosol TJ, Shah RB, Rehemtulla A, McCauley LK, Pienta KJ. 2003. In vivo visualization of metastatic prostate cancer and quantitation of disease progression in immunocompromised mice. Cancer Biol Ther 2:656–660.

Keller ET. 2002. The role of osteoclastic activity in prostate cancer skeletal metastases. Drugs Today (Barc) 38:91–102.

Lee D. 2003. Clinical trials of atrasentan in hormone-refractory prostate cancer. Clin Prostate Cancer 2:84–86.

Loberg RD, Fridman Y, Pienta BA, Keller ET, McCauley LK, Taichman RS, Pienta KJ. 2004. Detection and isolation of circulating tumor cells in urologic cancers: A review. Neoplasia 6:302–309.

Maragoudakis ME, Tsopanoglou NE, Andriopoulou P. 2002. Mechanism of thrombin-induced angiogenesis. Biochem Soc Trans 30:173–177.

Meerovitch K, Bergeron F, Leblond L, Grouix B, Poirier C, Bubenik M, Chan L, Gourdeau H, Bowlin T, Attardo G. 2003. A novel RGD antagonist that targets both alphavbeta3 and alpha5beta1 induces apoptosis of angiogenic endothelial cells on type I collagen. Vasc Pharmacol 40: 77–89.

Mohammad KS, Guise TA. 2003. Mechanisms of osteoblastic metastases: Role of endothelin-1. Clin Orthop 2:S67–S74.

Mundy GR. 2002. Metastasis to bone: Causes, consequences, and therapeutic opportunities. Nat Rev Cancer 2:584–593.

Nelson JB. 2005. Endothelin receptor antagonists. World J Urol 23:19–27.

Nelson JB, Nabulsi AA, Vogelzang NJ, Bruel J, Zonnenberg BA, Daliani DD, Schulman CC, Carducci MA. 2003. Suppression of prostate cancer induced bone remodeling by the endothelin receptor A antagonist atrasentan. J Urol 169:1143–1149.

Neves M, Gano L, Pereira N, Costa MC, Costa MR, Chandia M, Rosado M, Fausto R. 2002. Synthesis, characterization, and biodistribution of bisphosphonates Sm-153 complexes: Correlation with molecular modeling interaction studies. Nucl Med Biol 29:329–338.

Norton L, Simon R. 1977. Growth curve of an experimental solid tumor following radiotherapy. J Natl Cancer Inst 58:1735–1741.

Palmedo H, Manka-Waluch A, Albers P, Schmidt-Wolf IG, Reinhardt M, Ezziddin S, Joe A, Roedel R, Fimmers R, Knapp FF Jr, Guhlke S, Biersack HJ. 2003. Repeated bone-targeted therapy for hormone-refractory prostate carcinoma: Tandomized phase II trial with the new, high-energy radiopharmaceutical rhenium-188 hydroxyethylidenediphosphonate. J Clin Oncol 21:2869–2875.

Pantuck A. 2005. http://www.cancer.gov/clinicaltrials.

Posey JA, Khazaeli MB, DelGrosso A, Saleh MN, Lin CY, Huse W, LoBuglio AF. 2001. A pilot trial of Vitaxin, a humanized anti-vitronectin receptor (anti alpha v beta 3) antibody in patients with metastatic cancer. Cancer Biother Radiopharm 16:125–132.

Roudier MP, Vesselle H, True LD, Higano CS, Ott SM, King SH, Viessella RL. 2003. Bone histology at autopsy and matched bone scintigraphy findings in patients with hormone refractory prostate cancer: The effect of bisphosphonate therapy on bone scintigraphy results. Clin Exp Metastasis 20:171–180.

Rudek MA, Figg WD, Dyer V, Dahut W, Turner ML, Steinberg SM, Liewehr DJ, Kohler DR, Pluda JM, Reed E. 2001. Phase I clinical trial of oral COL-3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer. J Clin Oncol 19:584–592.

Saikali Z, Singh G. 2003. Doxycycline and other tetracyclines in the treatment of bone metastasis. Anticancer Drugs 14:773–778.

Saitoh H, Hida M, Shimbo T, Nakamura K, Yamagata J, Satoh T. 1984. Metastatic patterns of prostatic cancer. Correlation between sites and number of organs involved. Cancer 54:3078–3084.

Schellhammer PF, Hershberg RM. 2005. Immunotherapy with autologous antigen presenting cells for the treatment of androgen independent prostate cancer. World J Urol 23:47–49.

Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fatatis A. 2004. CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells. Cancer Res 64:4693–4698.

Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA. 2004. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 351:1502–1512.

Taranta A, Brama M, Teti A, De luca V, Scandurra R, Spera G, Agnusdei D, Termine JD, Migliaccio S. 2002. The selective estrogen receptor modulator raloxifene regulates osteoclast and osteoblast activity in vitro. Bone 30:368–376.

Tu SM, Millikan RE, Mengistu B, Delpassand ES, Amato RJ, Pagliaro LC, Daliani D, Papandreou CN, Smith TL, Kim J, Podoloff DA, Logothetis CJ. 2001. Bone-targeted therapy for advanced androgen-independent carcinoma of the prostate: A randomised phase II trial. Lancet 357:336–341.

Winquist E, Berry S. 2004. Re: A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. J Natl Cancer Inst 96:1183; author reply 1183–1184.

Withrington PG, de Nucci G, Vane JR. 1989. Endothelin-1 causes vasoconstriction and vasodilation in the blood perfused liver of the dog. J Cardiovasc Pharmacol 13(Suppl 5):S209–S210.

Yu H, Diamandis EP, Wong PY, Nam R, Trachtenberg J. 1997. Detection of prostate cancer relapse with prostate specific antigen monitoring at levels of 0.001 to 0.1 microG./L. J Urol 157:913–918.

Zonnenberg BA, Groenewegen G, Janus TJ, Leahy TW, Humerickhouse RA, Isaacson JD, Carr RA, Voest E. 2003. Phase I dose-escalation study of the safety and pharmacokinetics of atrasentan: An endothelin receptor antagonist for refractory prostate cancer. Clin Cancer Res 9:2965–2972.