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PROSPECTS

Modulation of Prostate Cancer Growth in Bone Microenvironments

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Abstract Bone remains one of the major sites, and most lethal host organs, for prostate cancer metastasis. Prostate cell spread and establishment in bone depends on multiple reciprocal modifications of bone stromal and epithelial cancer cell behaviors. This review focuses on recent advances in the characterization of cell–cell and cell–matrix interplay, effects on cell growth, adhesion and invasion, and several therapeutic possibilities for co-targeting prostate cancer cells and bone stroma. We address the topic from three main perspectives: (1) the normal and aging bone stromal environment, (2) the ''reactive'' bone stromal environment, and (3) the cancerous prostate epithelial cells themselves. First, normal, and especially aging, bones provide uniquely rich and ''fertile soil'' for roaming cancer cells. The interactions between prostate cancer cells and insoluble extracellular matrices, soluble growth factors, and/or sex steroid hormones trigger bone remodeling, through increased osteoclastogenesis and furthur matrix metalloproteinase activity. Second, after cancer cell arrival and establishment in the bone, host stromal cells respond, becoming ''reactive'' in a process again involving extracellular matrix remodeling, together with growth factor and steroid receptor signaling this process ultimately enhances cancer cell migration, stromal transdifferentiation, and invasion of the cancer tissues by stromal, inflammatory, and immune-responsive cells. Third, prostate cancer cells also respond to supportive bone microenvironments, where soluble and matrix-associated molecules affect cancer cell growth and gene expression, especially altering cancer cell surface receptor and integrin-mediated cell signaling. We discuss both integrin cell–matrix and gap junctional cell–cell communication between cancer cells and their microenvironments during prostate cancer progression. J. Cell. Biochem. 91: 686–705, 2004.

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Like many other metastasizing cancer cells, cancerous prostate epithelial cells interact with multiple distinct organ microenvironments, including the surrounding prostate gland before and during their escape, the lymphatic and vascular endothelia during transit, and the bone upon establishment within the skeleton. To successfully treat patients with prostate cancer bone metastasis and its associated complications, such as spinal cord compression, pathological bone fractures, chronic pain, anemia, and infection, a better understanding is needed of the interactions between prostate cancer cells and their environments, especially those of the bone. This review focuses on the recent characterization of this cell biology, in particular bone regulation of prostate cancer cell growth, adhesion and invasion, as well as the reciprocal effects of the stromal environment on cancer cells.

It has long been observed that tumors of different origins appear predisposed to metastasize to different organs, depending primarily on the circulatory system to gain access. In the 1800s, cancer cells were detected in the blood

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stream [Cruveilhier, 1829; Ashworth, 1869] and identified as metastatic emboli in the 1950s [Engell, 1955; Roberts et al., 1958]. As early as 1889, Dr. Stephan Paget [Paget, 1889] examined the autopsy records of 735 women who died of breast cancer and proposed the popular "seed and soil hypothesis," arguing that the tumor cells (the seeds) had specific affinities for the growth milieu provided by certain organs (the soil). More recent experiments have revealed that distinct locations of metastases can be predicted and traced following injection of melanoma cells into mice [Fidler and G, 1976]. The skeleton is fertile ground for many tumor types. Approximately 95–100% of myelomas, 65–75% of breast and prostate cancers, 60% of thyroid cancer, and 15–45% of bladder, lung, and renal cancers and melanomas end up in bone [Cifuentes and Pickren, 1979]. When considering skeletal metastasis, researchers have focused on such topics as: the tropism of cancer cells to bone, the roles played by the bone marrow's reticulate vasculature, its provision of uniquely rich oxygen, nutrients and survival factors to tumor cells, the routes taken by heterogeneous cancerous cell types, and their migration and invasion into marrow stroma, and finally, subtle differences between bone microenvironments. Prostate cancer cells may employ a unique portal-like venous drainage system between the prostate and the lower spine [Bastón, 1940; Resnick, 1992; Bubendorf et al., 2000]. The size and frequency of metastatic tumors found in 1,589 prostate cancer autopsies revealed that not only do spine metastases precede those to lung and liver, but there is an upward metastatic spread along spinal veins after initial lumbar metastatic deposits are established [Bubendorf et al., 2000]. Although many metastases remain unpredictable, suggesting complex and multistep mechanisms for cancer cell spread and establishment [Liotta and Kohn, 2001], bone remains the major organ for prostate cancer metastasis. This review is subdivided into three parts: (1) the normal and aging bone stromal environment as a "rich soil," (2) the reactive, or activated bone stromal environment and its effects on the interplay between prostate cancer and bone stromal cells, and (3) the responsive character of prostate cancer cells, themselves, especially in terms of integrin-mediated cell–matrix, and gap junction-mediated cell–cell communications.

NORMAL AND AGING BONE STROMA: A RICH SOIL FOR CANCER CELLS

Bone heterogeneity and the complexity of microenvironments are key to an understanding of restriction and selectivity in cancer cell spread within a non-uniform environment. Prostate cancer cells are known to "prefer" trabecular bone, also called spongy or cancellous bone, a bone type composed of a network of tiny strands of bone trabeculae. Such bone is found at the ends of long bone, in ribs, in the pelvis, vertebrae, and the skull and is interspersed with marrow and a rich vasculature. For successful metastasis, the cells of trabecular bones must attract cancerous prostate cells, allow them entry into the tissue, and provide optimal growth conditions once inside.

Entry into the bone marrow is generally believed to depend upon the arrest of circulating cancer cells in the vascular beds, with single cells arresting in the capillaries, while multicellular aggregates arrest in larger vessels [Liotta et al., 1976]. Recent evidence argues that only endothelium-attached cancer cells are able to give rise to metastases [Al-Mehdi et al., 2000]. Cell "docking" on the vasculature involves recognition of protein receptors, such as selectins, integrins, cadherins, and immunoglobulin superfamily members. These proteins may be expressed initially by the cancer and/or vascular endothelial cells in response to disease progression and local cues, such as growth factors, cytokines, chemokines, or reactive oxygen species. Along with the heterotypic cell interactions between cancer cells and endothelia, homotypic interactions within multicellular cancer cell aggregates also occur. In several instances, in vivo selection for tumor cells of high metastatic potential has resulted in the selection of cells with increased homotypic aggregation properties [Saiki et al., 1991; Glinsky and Glinsky, 1996]. This correlation appears to depend upon selectins and β-galactoside-mediated adhesive interactions, at the sites of primary attachment to the microvascular endothelia [McEver, 1997; Glinsky et al., 2003]. Beyond specific cell adhesions, bone endothelial sites may also be favored for biomechanical reasons. Unlike other bones, the vascular beds within trabecular bones form sinusoids of large diameter and reduced blood flow rate (up to ten times slower), possibly allowing increased cancer cell attachment to

vessel surfaces, and their access across only a few microns into the stromal marrow compartment itself [Schnitzer et al., 1982]. This unique vasculature may help to resolve conflicting results from calculations of the times required for sufficient cancer cell attachments to endothelia prior to appearance in the bone [Haier and Nicolson, 2001a,b].

A prostate epithelial cells ability to intercalate among bone cell's may be of greater importance than the strength of their initial adhesion; in vitro, prostate carcinoma cells preferentially interact with bone marrow endothelial cells (HBME), adhering to HBME over umbilical vein, aortic, dermal, or lung endothelial cells (HUVEC, HAEC-1, HDMVEC, and Hs888Lu, respectively) [Lehr and Pienta, 1998; Scott et al., 2001; Sikes et al., 2003 submitted]. Shortly after prostate (and breast) cancer cells relocate to vessel walls, they express factors that are known to stimulate endothelial retractions and are believed to induce "reactive" stroma. One such factor is osteonectin. This small Ca^{2+} ion-binding glycoprotein is normally expressed by osteoblasts, endothelial cells, and megakaryocytes [Rodan and Noda, 1991; Kelm et al., 1992; Thomas et al., 2000; Brekken and Sage, 2001; Lin et al., 2001]. Cancer cell expression of osteonectin is involved in Human Epidermal growth factor Receptor 2 (HER2, also referred to as Her2/neu or c-erbB2) tissue remodeling and the breaching of vessel walls [Sanchez et al., 2002; Holbro et al., 2003]. Porous vessel walls give the cancer cells access to both chemotactic factors from the underlying tissue compartments and new extracellular matrices.

Once attached, cancer cells encounter varying levels of chemotactic, adhesion, and growth factors provided by marrow stromal cells [Hart, 1982; Liotta and Kohn, 2001]. Not only do prostate cancer cells adhere to bone marrow endothelial cells, osteoblasts, and prostate stromal fibroblasts [Haq et al., 1992; Lehr and Pienta, 1998], but media conditioned by these cells can stimulate prostate cancer cell growth [Lang et al., 1995]. Further evidence that bone stromal cells may prepare their soil with unique, even species-specific "fertilizers" comes from Tsingotjidou et al. [2001], who demonstrated that human prostate cancer cells home to experimentally implanted human bone, rather than to the mouse bone within their athymic mouse hosts. The identities and roles of the released factors responsible for such homing are not yet clear [Tsingotjidou et al., 2001].

A recurring statement in the literature is that bone matrix is composed of more than 90% type I collagen fibrils secreted by osteoblasts, but bone is a dynamic tissue, constantly being remodeled and altered through both local and systemic cues. Maintenance of compartmentalization and multiple delicate balances in collagenous and non-collagenous components of the bone extracellular matrix are key to tissue homeostasis, bone remodeling, and disease, but have proven difficult to study, as they depend upon many cell types and regulation by cytokines, growth factors, and matrix metalloproteinases. Isotype-specific collagen antibodies have revealed that collagens are compartmentalized. Although collagens I, III, and X are all matrix-localized, collagen III is the most abundant in the marrow [Reddi et al., 1977], where it is produced by cells of fibroblast lineage [Castro-Malaspina et al., 1980]. Type IV collagen is produced by endothelial cells [Jaffe et al., 1976] and is found in the basement membranes of the vascular sinuses [Gay et al., 1984], where the endothelial cells of the vessel walls are separated from the adventitial cells by the collagen IV and laminin basement membrane [Inoue and Osmond, 2001]. Likewise, non-collagenous matrix components, including fibronectin, vitronectin, laminin, and bone sialoprotein, may also be compartmentalized and shift levels or localization during bone remodeling or cancerous invasion. For example, laminin 5 (LM-5) protein occurs in a novel bone-specific isoform (with the chains $\alpha 5$, $\beta 2$, and $\gamma 2$) in multiple splice versions, and is markedly absent in cancerous prostate cells before their establishment in bone [Siler et al., 2000, 2002; Hao et al., 2001].

Much of the bone matrix is produced by osteoblasts, whose activities are closely coupled to those of the osteoclasts and invading cancer cells. In vitro osteoblasts are known to be able in the presence of ascorbic acid and β-glycerophosphate, to form a mineralized bone matrix closely resembling woven bone [Quarles et al., 1992]. Metastasizing prostate cancer cells, once inside the bone, actually mimic osteoblasts, not only in their synthesis and deposition of non-collagenous bone matrix proteins (including osteopontin, osteonectin, osteocalcin, and bone sialoprotein) [Koeneman et al., 2000], but also by participating directly in osteoclastogenesis

and the formation of mineralized bone. This ability to take part in osteoclastogenesis has been linked to prostate epithelial cell expression of RANKL (receptor activator of NF-κB ligand) and sRANKL (soluble RANKL), both of which are capable of activating osteoclasts through the cell surface receptor, RANK [Boyle et al., 2003; Lee et al., 2003]. Activated osteoclasts degrade the bone, releasing a number of crucial growth factors and cytokines, including TGF-β, IGF-1, and IL-6, and directly or indirectly promoting proliferation of both osteoblasts and prostate cancer cells [Brown et al., 2001a,b]. Together, invading prostate cancer cell matrix deposition and growth factor regulation are likely to have profound effects on cell adhesion, motility, proliferation, and survival.

Given that bone stromal-derived growth factors affect cancer cell response to chemokines, in a trans-activation process [Mira et al., 2001], a better understanding is needed of both systemic and local cues for bone remodeling, as well as shifts in bone microenvironmental composition, especially in the context of aging bone. It is generally accepted that bone turnover rates and bone loss are accelerated in response to shifts of hormone level, but there are differences in bone loss at different skeletal sites and between cortical and trabecular bone. Aging is accompanied by a reduction in both trabecular bone volume and numbers of endosteal and osteocyte cells. Additionally, hematopoietic tissue decreases, while fat cells increase. Although there is great variability in the scale of these changes, depending upon both sex and age, two mechanisms involving matrix proteins have been outlined to explain them. The first is based upon the extracellular matrix becoming more rigid, due to cross-linking of matrix proteins and changes in surface charge, either by glycation or by oxidation of lysyl and hydroxy lysyl residues to aldehydes [Miyata et al., 1997; Bailey et al., 1998; Hadley et al., 1998]. These changes are accompanied by degradation of the collagen and other matrix proteins [Termine, 1990]. The second mechanism involves decreased production of the most common matrix proteins, as seen in cells from donors of increasing age [Fedarko et al., 1990, 1992, 1995]. Such decreases are likely to alter the soluble growth factor and cytokine cues normally contained within the matrix.

The intersection between research findings on hormones, bone remodeling, aging, and prostate cancer is complex, but rich, especially because of well known hormonal effects on extracellular matrix composition, growth factors, and cytokines. Balances of estrogenic and androgenic actions on bone have been studied in animal models, where estrogen functions to reduce longitudinal growth rate, whereas androgens actually stimulate chondrocyte maturation and metaphyseal ossification [Lebovitz and Eisenbarth, 1975]. Although androgen deficiency can reduce these processes, androgen elevations are also able to reduce growth, possibly indirectly, through aromatization to form estrogen [Iannotti, 1990; Hermann et al., 2000]. The increased estrogen levels observed in obese patients, together with altered metabolism in the fat cells, likely support transdifferentiation of prostatic fibroblasts into smooth muscle cells, an important event early in the development of benign prostatic hyperplasia (BPH), among aging males. Increases in femoral fracture incidence, which also correlate with aging, may be at least partly attributable to shifts in the rates of bone turnover, although changes in bone mass are not as well documented [Boonen et al., 1997]. While some have correlated androgen levels inversely with bone loss, others have disputed these findings and claim that bone mass is unrelated to serum testosterone and adrenal androgens.

In addition to circulating sex steroids, some cytokines directly regulate bone cell behavior, while others appear to act indirectly through growth factors. Shifts in both cytokine and growth factor levels are known to accompany aging. For example, interleukin-6 (IL-6) levels increase in the serum of older individuals, particularly after menopause or andropause. IL-6 not only regulates inflammation, but has been implicated in rheumatoid arthritis, osteoporosis, multiple sclerosis, and most notably has been identified as a direct regulator of osteoblast function [Gimble et al., 1994; Jay et al., 1996]. Other factors linked to aging and changing estrogen levels are interleukin-1 (IL-1) and tumor necrosis factor (TNF-α). IL-1 and TNF-α are among the most powerful stimulators of bone resorption known, and well-recognized inhibitors of bone formation. However, systemic levels should not be considered the only measures of a regulator's effectiveness. Local concentrations need not necessarily change as systemic levels do, and osteoblast responses to growth regulators may shift as well. Such is the case for osteoblast response to insulin-like

growth factor-1 (IGF-1). IGF-1 receptor levels do not change, but osteoblast response to the growth factor decreases with age [Pfeilschifter et al., 1993, 2000; D'Avis et al., 1997; Ankrom et al., 1998]. In this way, age-associated shifts in any given factor could be either further enhanced or ameliorated quite locally [Rosen, 2000].

TUMOR-ASSOCIATED STROMA: ACTIVE PARTICIPANTS IN CANCER PROGRESSION

Stromal cells are now known to actively modify cancer cell behavior, instead of providing only passive support for local cancer growth. In fact, cancer and stromal cells reciprocally modify each other's microenvironments, using feedback cycles to support cell growth and differentiation, during both normal development and disease [Chiquet-Ehrismann et al., 1986; Yee et al., 1989; Basset et al., 1993; Wright et al., 1994; Singer et al., 1995]. In the LNCaP human prostate cancer progression model, we demonstrated a change in cell phenotype from marginally tumorigenic behavior to tumorigenic and bone metastatic behavior, following cell following cell co-culture with other human prostate or bone stromal cells in vitro [Rhee et al., 2001] and in vivo [Thalmann et al., 1994]. Organ-specific stromal cell treatment can irreversibly determine genomic organization of cancer epithelia, resulting in cancer cell progression to androgen independence and increased bone metastatic potential [Thalmann et al., 1994, 2000].

Fibroblasts and smooth muscle stromal cells in the human prostate gland synthesize both the structural and regulatory components of the extracellular matrix, forming a meshwork of fibrillar, adhesive glycoproteins, and proteoglycans. This matrix serves as a reservoir of active and latent growth factors [Tuxhorn et al., 2001], such that stromal cells are greatly affected by matrix remodeling during cancer progression. In prostate and other cancers, stromal cells at the invasion fronts have been shown to change both morphologically and biochemically, converting from fibroblasts to myofibroblasts, and expressing vimentin, versican, hyaluronic acid, MMP2, MMP9, fibroblast activation protein (FAP), pro-collagen Type I, and tenascin [Tuxhorn et al., 2002a,b,c]. These changes are termed stromal activation, and correlate negatively with patient survival [Tuxhorn et al., 2001, 2002a]. Conversion to myofibroblasts occurs in colon, liver, lung, breast, pancreas, and prostate cancers, where it is localized to the invasion fronts [Miura et al., 1993; Neaud et al., 1995; Ronnov-Jessen et al., 1995; Rowley, 1998; Doucet et al., 2000; Lohr et al., 2001]. In breast cancer, myofibroblast progenitors have been identified as fibroblasts (100%), vascular smooth muscle cells (40%), and pericytes (10%) [Bissell et al., 1999; Bissell and Radisky, 2001]. In the last case, pericytes would need to migrate from the blood vessel basement membrane to the interstitial collagenous stroma [Ronnov-Jessen et al., 1995].

Thus, when activated, stromal cell populations change both phenotypically and genotypically [Moinfar et al., 2000], but questions remain about how many of the stromal myofibroblasts are endogenous (but altered by association with the cancer cells) and how many are "recruited" from other cellular compartments. A three-dimensional co-culture study in our program, using multiple stromal cell lines (the human osteosarcoma cell line MG-63, a human prostatic stromal cell line, and the marginally tumorigenic LNCaP cell line), resulted in permanent, non-random genotypic changes of the LNCaP cells, together with associated phenotypic changes in tumorigenicity and metastatic potentials [Rhee et al., 2001]. The reciprocal is also true, as androgen-independent, metastatic C4-2 tumor cells can genotypically and phenotypically alter co-cultured stromal cells (Sung et al., unpublished observation). These in vitro studies effectively argue for transdifferentiation of pre-existing, tumor-associated stroma, rather than cell recruitment during cancer progression.

Molecular Regulation of Stromal Conversion

Myofibroblast conversion in primary prostate tissue can be regulated in vivo and in vitro by transforming growth factor β1 (TGF-β1) [Tuxhorn et al., 2002a,b,c], with necessary TGF-β1 concentrations decreasing as prostate cancer cell malignancy status increases [Tuxhorn et al., 2002a,b,c]. As a candidate regulator for the genesis of reactive stroma at Prostatic Intraepithelial Neoplasia (PIN) sites, TGF-β1 increase in high-grade PIN lesions [Tuxhorn et al., 2002a,b,c]. TGF-β1 regulates the stromal response to wound repair, a response commonly compared to tumor progression (see below), and is also known to regulate angiogenesis. When TGF-β1 expression is blocked, angiogenesis

decreases dramatically [Tuxhorn et al., 2002a,b,c]. This effect may be linked to myofibroblast expression of vascular endothelial growth factor (VEGF), in response to hypoxia or other growth factor release [Orlandini and Oliviero, 2001]. In fact, TGF-β1 is known to act not only with VEGF, but also platelet derived growth factor (PDGF), and fibroblast growth factor (bFGF) to drive the reactive stromal response during cancer progression [Tuxhorn et al., 2001; Sung and Chung, 2002; De Wever and Mareel, 2003]. TGF-β1 is secreted as a latent complex that is subsequently activated by proteinase cleavage, involving furin, plasmin, cathepsin, human mast cell chymase, leucocyte elastase, matrix metalloproteinases MMP2, MMP9, MT1-MMP, $\alpha_v\beta_6$, and $\alpha_v\beta_8$ integrins, thrombospondin 1, and environmental pH change [Lyons et al., 1990; Taipale et al., 1995; Crawford et al., 1998; Munger et al., 1999; Yu and Stamenkovic, 2000; Mu et al., 2002].

Because TGF-β1 receptors are down-regulated in carcinoma cells, but remain high in stromal cells, TGF-β1 is thought to act directly on stroma [De Wever and Mareel, 2003]. Despite the many factors that have now been shown to act during TGF-β1 regulation of stroma, downstream responses within the stromal cells are less well understood. TGF-β1 treatment does appear to affect the smads pathways. Smads are named after *C. elegans* Sma and *Drosophila* Mad proteins, the first identified members of this class of signaling effectors, and are known to increase production of fibronectin, plasminogen activator I, and cyclin-dependent kinase (CDK) inhibitors [De Wever and Mareel, 2003]. The TGF-β1 signal pathway is also linked to the MAPK pathways, which in turn affect other regulators, including extracellular regulated kinase (ERK), c-jun amino-terminal kinase (JNK), and p38 [Akhurst and Balmain, 1999; ten Dijke and Heldin, 1999].

The Shifting Balance Between Osteoblasts and Osteoclasts

Bone extracellular matrices contain many embedded factors that are released when the balance of osteoblast and osteoclast functions is shifted and the matrix remodeled. Prostate cancer skeletal metastases are characterized primarily as osteoblastic with an underlying osteoclastic component [Keller, 2002]. Advances in the understanding of osteoclastogenesis and prostate cancer bone metastasis have defined several promising therapeutic targets for reduction of tumor-induced osteolysis. One factor found to be important in tumor-induced promotion of osteoclast activity is the RANKL protein (mentioned above; Table I). The soluble form of this protein (sRANKL) is required during normal osteoclastogenesis, but both RANKL and sRANKL are produced by prostate cancer cells within the bone, where they activate osteoclasts and induce osteolysis. Another factor, osteoprotegerin, a soluble decoy receptor for RANKL, inhibits RANKL-induced osteoclastogenesis [Zhang et al., 2001], and in murine models, has been found to inhibit tumor-induced osteolysis. Parathyroid hormone-related protein and interleukin-6 (IL-6) are also produced by prostate cancer cells and can promote osteoclastogenesis (Table I). Other key factors orchestrating stromal reactions are TGF-β1 [Tuxhorn et al., 2002a,b,c], platelet-derived growth factor (PDGF); [Roodman, 2003], and factors responsible for activation of latent TGF-β1 (such as matrix metalloproteinases) or co-signaling within the TGF-β1 pathway (such as Wnt-catenin and Ras); [Rowley, 1998]. Drugs that specifically inhibit TGF-β1′s activities are particularly promising; these include soluble TGF-β1 antagonists, TGF-receptor antagonists, inhibitors of TGF-β1 intracellular signaling, and metalloproteinase inhibitors that block latent TGF-β1 activation [De Wever and Mareel, 2003].

Matrix metalloproteinases (MMPs) warrant special attention in the context of bone remodelling, as these zinc-dependent proteinases are secreted by prostate cancer cells and are known to promote osteolysis by degrading bone matrix [Nemeth et al., 2002]. MMP2 and MMP9 have both been associated with prostate cancer [Dong et al., 2001; Nemeth et al., 2002] and are found at increased levels in the blood plasma and urine of patients with prostate metastases [Gohji et al., 1998; Moses et al., 1998]. Membrane-bound proteinase MT1-MMP levels also increase during progression from benign prostate disease to PIN, and further to cancer [Upadhyay et al., 1999; Nagakawa et al., 2000; Udayakumar et al., 2003]. MT1-MMP is involved in the proteinase activation of pro-MMP-2, pro-collagen 1, and laminin [Ohuchi et al., 1997; Udayakumar et al., 2003]. Matrix metalloproteinases are also active during osteoclast recruitment to sites of

TABLE I. Secreted Factors From Prostate Epithelial Cells That Stimulate Bone Remodeling by Modulating Osteoblast and Osteoclast Activity

Factors	Bone formation	Bone resorption
Bone resorption		
sRANKL		+(1)
Osteoprotegerin (OPG)		+(1)
IL-1		+(2)
Cathepsin K		+(3)
Collagen I amino-terminal telopeptide (NTX)		+(4)
α-Collagen I carboxy terminal telopeptide (α-CTX)		+(4)
β-Collagen I carboxy terminal telopeptide (β-CTX)		+(4)
Collagen I cross-linked carboxy terminal telopeptide (ICTP)		+(4)
TNF-α		+(2)
ET-1		−(5)
Bone formation		
Galectin-1	+(6)	
Cyclophilin A	+(7)	
BMP-2	+(2)	
BMP-4	+(2)	
BMP-6	+(8)	
Bone alkaline phosphatase (BAP)	+(4)	
Pro-collagen I amino-terminal propeptide (PINP)	+(4)	
ET-1	+(9)	
PTHrP	+(10)	
IL-6	+(2, 11)	
Placenta bone morphogenic protein (PLAB)	+(12)	
Bone sialoprotein (BSP)	+(13)	
Osteoprotegerin (OPG)	+(2)	
IGFBP-3	−(14)	

(1) [Brown et al., 2001a,b], (2) [Lee et al., 2003], (3) [Brubaker et al., 2003], (4) [de la Piedra et al., 2003], (5) [Chiao et al., 2000], (6) [Andersen et al., 2003a], (7) [Andersen et al., 2003b], (8) [Autzen et al., 1998], (9) [Guise et al., 2003], (10) [Dougherty et al., 1999], (11) [Garcia-Moreno et al., 2002], (12) [Thomas et al., 2001], (13) [Waltregny et al., 2000], (14) [Fizazi et al., 2003].

bone remodeling [Keller, 2002; Nemeth et al., 2002].

Both mineralized and non-mineralized bone matrices are affected by a variety of osteoclast-associated enzymes, including cysteine proteinases and MMPs. A number of synthetic MMP inhibitors are able to slow bone resorption in bone culture systems, including chemical inhibitors of MMP-2 and MMP-9 [Hill et al., 1994, 1995]. If a "vicious cycle" is initiated during prostate cancer metastasis, such that cancer cells stimulate bone matrix turnover, which in turn stimulates cancer growth [Nemeth et al., 2002], MMPs may make ideal therapeutic targets for the cycle's disruption. In murine models, MMP inhibitors have been shown to diminish tumor establishment in bone. The regulation of MMP activation and activity is strongly dependent on the levels of Tissue Inhibitor of MetalloProteinases (TIMPs); [Liotta et al., 1991; Nagase, 1997; Brew et al., 2000]. Hence, balance between proteinases and inhibitors may determine the net enzymatic activity present in the tissue. Significant down-regulation of TIMP1 and TIMP2 correlate with the induction of pro-MMP9 expression, in co-culture of prostate cancer and stroma [Dong et al., 2001]; this effect is likely due to the stromal cells, as they are the major producers of TIMPs. Cancerous progression could, then, be supported by both increased MMP expression and decreased TIMPs, and the resultant changes in MMP concentrations and half-lives. Other promoters of osteolysis, derived from prostate cancer metastases, may now also be considered as new therapeutic targets [Dong et al., 2001].

Stromal Invasion of Epithelia: Wounds That do not Heal

During organogenesis and normal maintenance of adult organs, stromal and inflammatory cells intercalate among epithelial cells, regulating morphogenesis and cytodifferentiation [Ronnov-Jessen et al., 1996; Bissell and Radisky, 2001]. As active participants in carcinogenesis, stroma can: (1) increase deposition of extracellular matrices [Sung and Chung, 2002], (2) recruit other "reactive" stromal fibroblasts or myofibroblasts to the tumor [Tuxhorn et al., 2002a,b,c; Zidar et al., 2002], and (3) alter microenvironements adjacent to the tumor, through inflammatory and immune-responsive cell secretion of cyto- and chemokines [Svennevig, 1980; Heinrich et al., 2003; Wang et al.,

2003]. Because this sequence of events mimics classic wound-healing cascades (albeit without normal levels of apoptosis), cancers have been referred to as "wounds that do not heal" [Tuxhorn et al., 2001, 2002a]. In this context, the stromal reaction to tumor epithelia could be viewed as a host defense mechanism, initially intended to curtail or restrict tumor expansion. Sadly, the stromal reaction ultimately increases stromal cell number, alters stromal differentiation, and produces the extracellular matrices, growth factors, and metalloproteinases of a uniquely "fertile soil" for the support of tumor cell growth and invasion.

THE CANCER CELL: A MOVING TARGET

Not only the bone stromal cells, but also the prostate cancer cells change irreversibly during disease progression and metastasis. Because of feedback loops, it is difficult to separate early soil changes from those in the seeds. As human prostate carcinoma cells change phenotype they actively participate in bone turnover, thus affecting both the stroma and their own subsequent regulation. Attempted time-courses and causal relationships are often unclear on whether shifts in protein expression by the prostate cells occur before extravasation or are the consequence of tumor microenvironment interaction at the new location. To obtain a true developmental time-course, one would have to analyze the circulating prostate cells in patients before metastases occur [Ts'o et al., 1997; Wang et al., 2000; Ellis et al., 2003] and compare expression profiles of prostate cancer cells from primary tumors and marrow biopsy specimens. Unfortunately, such measurements have not yielded clinically significant results, suggesting that the location of the prostate cancer cells may not be as telling as the cells' specific phenotypes. For example, over 50% of all prostate patients with clinically localized disease have prostate cells in their bone marrow, but only 10–30% are expected to develop recurrence after radical prostatectomy, and even fewer to develop detectable skeletal metastases. Thus, it seems likely that proliferative cancer cell phenotypes principally develop *after* re-localization to the bone environment.

Adhesion to Extracellular Matrices at Sites of Metastasis

Cancer cell adhesion to new extracellular matrices depends upon a variety of cell membrane receptors, including the integrins. Previous studies of integrin expression in various epithelial carcinomas have found that increased levels of the $\alpha_v\beta_3$ integrin heterodimer correlate well with metastatic potential, but otherwise many published observations conflict. Table II shows that integrin expression can change transiently during disease progression; thus, some of the published discrepancies may not

TABLE II. Differences in Integrin Expression Between Cell Lines of Different Metastatic Potential

Integrin	Cell line DU-145	PC3	LNCaP	RWPE-1	Change in carcinoma	Change in metastasis
$\alpha_2\beta_1$		0 (2)	+(3)		−(6)	+(6)
$\alpha_3\beta_1$		−(2)	+(3)		0 (7)	
$\alpha_5\beta_1$		0 (2)				
$\alpha_6\beta_1$	+(1)		+(3)		0 (7–9); +(6)	+(6)
$\alpha_6\beta_4$	+(1)	+(2)	−(3)		−(8–11)	
$\alpha_v\beta_3$		0 (2)	+(3)		+(12)	
α_2			+(4)		−(7)	
α_3	0 (1)		+(4)		−(7)	
α_4					− (7)	
α_5	0 (1)		+(4)		−(7)	
α_6	+(1)		0 (4)	− (5)	0 (13); −(7)	
α_v					−(6)	
β_1	0 (1)		+(4)		0 (13,14); − (7); +(15)	
β_{1A}					0 (16)	
β_{1C}					−(14,16,17)	
β_2					0 (7)	
β_3					0 (7)	+(18)
β_4			+(4)		−(7,9,10,11,13)	

+, increases; 0, stable; −, decreases. (1) [Rabinovitz et al., 1995], (2) [Dedhar et al., 1993], (3) [Edlund et al., 2001], (4) [Freedland et al., 2003], (5) [Bello-DeOcampo et al., 2001], (6) [Bonkhoff et al., 1993], (7) [Cress et al., 1995], (8) [Nagle et al., 1994], (9) [Nagle et al., 1995], (10) [Allen et al., 1998], (11) [Davis et al., 2001], (12) [Zheng et al., 1999], (13) [Knox et al., 1994], (14) [Perlino et al., 2000], (15) [Murant et al., 1997], (16) [Fornaro et al., 1996], (17) [Fornaro et al., 1999], (18) [Hartstein et al., 1997].

only be attributed to differences in methodology and antibodies, but to cell stage. In prostate cancer research, one α integrin subunit, α6, has received the majority of attention. The α6 subunit pairs with β_1 or with β_4 in hemidesmosomal structures. β_4 is not found in metastatic cells [Allen et al., 1998], whereas α_6 expression decreases more slowly during disease progression, and actually increases remarkably in lymph node metastasis [Bonkhoff et al., 1993]. This pattern is somewhat similar to that of α_2, although decreases in α_2 are detectable at much earlier stages. Further complexity in protein pattern was revealed by findings in the LNCaP prostate cancer progression system; we discovered that as prostate cancer cells progress, differences in the regulation of integrin subunit usage and heterodimerization are much more marked than differences in the surface expression of most integrins [Edlund et al., 2001].

Unlike the other integrin heterodimers, expression and activation of the vitronectin receptor $\alpha_v\beta_3$ integrin has been clearly linked to the metastasis and progression of many cancers [Juliano and Varner, 1993], most specifically to cell migration through basement membranes, in studies using function-blocking antibodies in vivo [Brooks et al., 1994a,b]. $\alpha_v\beta_3$ integrin is also expressed by proliferating vascular endothelial cells during angiogenesis [Brooks et al., 1995; Drake et al., 1995]. Monoclonal antibodies or small molecular antagonists for $\alpha_v\beta_3$ are known to inhibit blood vessel formation (including tumor angiogenesis) in a variety of in vivo models [Eliceiri and Cheresh, 2000, 2001]. These promising results, however, are confounded by the finding that mice lacking both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ not only support enhanced tumor growth, but also present increased neovascularization [Reynolds et al., 2002].

Despite probable redundancy during angiogenesis and extravasion, antibodies, peptides, and chemical compounds have all been tested as integrin antagonists, with clinically significant results in some cases [Gutheil et al., 2000]. Furthermore, small, non-peptide inhibitors of both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin heterodimers inhibit tumor angiogenesis [Kumar et al., 2001; Kumar, 2003], and in our hands have successfully blocked previously established tumor growth in the femur, when systemically delivered (unpublished observation). The actual mechanism behind integrin-based decreases in tumor growth remains unclear, but could be direct regulation of cell proliferation and/or indirect regulation involving growth factors and angiogenesis. One intriguing indirect possibility is the cooperation between integrins and matrix metalloproteinases (MMP's). The binding of MMP-2 to integrin $\alpha_v\beta_3$ in invasive cells appears to act together with MT1-MMP to present activated MMP-2 on the cell surface [Hofmann et al., 2000]. Inhibitors used to block β_3-integrin function also inhibit MMP-2 by decreasing the expression of MT1-MMP [Pasco et al., 2000].

In addition to the integrins, Ig-domain cell adhesion molecule L1 (L1CAM) is also expressed in neuroblastoma, melanoma, and several carcinomas [Gabrielsen et al., 1988; Linnemann et al., 1989; Reid and Hemperly, 1992; Pancook et al., 1997]. L1 was originally identified as a neural cell adhesion molecule involved in axon guidance and neuronal migration [Fransen et al., 1997, 1998; Kadmon and Altevogt, 1997; Hoffman, 1998; Bliss et al., 2000; Thelen et al., 2002]. L1 protein interacts directly with both $\alpha_v\beta_3$ integrin and the fibronectin receptor, integrin $\alpha_5\beta_1$, as well as the cytoskeletal linker protein ankyrin [Bennett and Chen, 2001]. When expressed in the seminal vesicles and prostate of the urogenital tract [Kujat et al., 1995], L1 is normally restricted to non-proliferating epithelial cells. However, gene expression profiling in transgenic mouse models implicates L1 in both prostate and breast cancer cell progression to metastasis [Gutwein et al., 2000; Calvo et al., 2002]. Similarly, the L1 expression profiles of commonly used prostate cancer cell lines also correlate with high metastatic potential (Dr. Chia-Ling Hsieh, personal communication). Although normal prostatic tissue does not detectably express L1, the protein is readily found by immuno-staining in the stromal matrices adjacent to prostate tumors. L1's functions are still unknown, but interestingly, ARCaP cell expression and shedding of L1 correlates with the cell line's ability to penetrate endothelial monolayers [Zhau et al., 1996]. L1 likely influences epithelial–endothelial interactions and/or decreases vessel wall rigidity.

Altered Extracellular Matrix

Once in new environments, cancer cells not only bind extra-cellular matrix differently, but are known to alter the extracellular matrix adjacent to prostate, breast, and colon carcinomas, a key behavior in cancer

progression [Redler and Lustig, 1968, 1970]. Distal and proximal stromal fibroblasts differ from one another in adhesion, migration, and growth factor receptor expression. Likewise, production of woven bone (with random, loosely packed collagen strands) increases in prostate cancer patients relative to that of lammelar bone [Boyce et al., 1999]. These changes are regulated by secretion of osteoblastic factors by the prostate cells, regulating the core binding factor-1 (Cbfa1), a transcription factor known to control expression of osteocalcin, bone sialoprotein (BSP), osteopotin, and type I collagen in vivo [Ducy, 2000; Karsenty, 2000; Lin et al., 2001; Yang et al., 2001]. In vitro expression of antisense oligo-nucleotides for Cbfa1 decreases expression of these same osteoblastic genes [Banerjee et al., 1997; Ducy et al., 1997]. A similar transcription factor interruption strategy may be applicable against prostate cancer because of the cells' osteomimicry [Koeneman et al., 2000].

Cbfa1 affects Map Kinase (MAPK) and cyclic AMP (cAMP) signaling pathways, which in turn feedback on Cbfa1 protein levels. MAPK appears to activate Cbfa1, while cAMP reduces Cbfa1 protein levels [Xiao et al., 1998; Tintut et al., 1999; Xiao et al., 2000]. Cbfa1's actions on the MAPK pathway may involve other known MAPK stimulatory factors, including epidermal growth factor (EGF), fibroblast growth factor (FGF), and integrin adhesion molecules. In vitro, Collagen I has been found to regulate Cbfa1 [Xiao et al., 1998]. Not only does long-term cell culture on collagen I substrata induce osteoblastic differentiation of bone marrow cells, but this induction is abolished by addition of integrin function-blocking, collagen-specific peptides. Regulation of integrin–collagen interactions may be quite complex, with cryptic sites in the collagen molecules becoming exposed during matrix remodeling [Petitclerc et al., 2000; Xu et al., 2001], together with altered secretion levels of collagens I and III [Klein, 1995; Billiard et al., 2003].

Once cancer cells enter the bone microenvironment, one of the mechanisms used by cancer cells to modify their surroundings and regulate their own phenotype may be the shedding of membrane vesicles. Shed membrane vesicles are implicated in cancer cell escape from immune responses, the induction of angiogenesis, and the spread of metalloproteinase activity [Wood et al., 1997; Kim et al., 2002]. Membrane vesicles, derived of specific regions of the plasma membrane [van Blitterswijk et al., 1979, 1982; Lerner et al., 1983] and enriched in tumor cell surface antigens are shed both in vitro and in vivo. Interestingly, vesicle quantities in patient serum appear to correlate with cell invasiveness and tumor load [Dolo et al., 1994, 1995, 1998; Ginestra et al., 1998, 1999; Dolo et al., 1999].

Chemotactic Factors

In addition to osteonectin and osteopontin (the bone matrix protein scaffolds bound by $\alpha_v\beta_3$ integrins during migration; van der Flier and Sonnenberg, 2001), chemokines are small molecular weight cytokine-like peptides known to affect cytoskeletal arrangement and induce directional cell migration. Many chemokines are secreted, and more than 20 receptors identified. The 50 chemokines so far identified in humans are all structurally related, mostly basic, small (8–14 kDa) molecules, whose receptors form a large family of seven-pass transmembrane, G protein-coupled molecules. These receptors show considerable overlap in ligand specificity, and act in a complex regulatory network [Muller et al., 2001; Taichman et al., 2002; Pfitzenmaier et al., 2003; Sun et al., 2003]. Chemokines were originally found to act during the directed movement of leukocytes across the endothelial layers of blood vessels into tissue [Muller et al., 2001]. Both bone and lung stromal cells secrete the chemokine CXCL12, whose receptor CXCR4 is expressed on the cell surfaces of many breast tumor cells. Antibodies against this CXCR4 receptor are able to significantly reduce breast cell metastasis to regional lymph nodes and lung [Muller et al., 2001]. Expression levels of both CXCL12 (also called chemokine stromal factor-1, SDF-1) and its receptor were found to be elevated in localized and metastatic cancers [Sun et al., 2003], where such chemotactic factors could play key roles in directing cancer cell relocalization. Further studies are needed to assess whether levels of the same chemotactic factors that stimulate metastasis to the bone are altered physiologically during aging.

Gap Junctional Communications Among and Between Epithelial and Stromal Cells

Assessments of the mechanisms of bi-directional cell–cell communication—from stromal cell to cancer cell and cancer cell back to stromal cell [Gleave et al., 1991; Chung, 1993, 1995;

Sokoloff et al., 1996; Rowley, 1998; Tuxhorn et al., 2001, 2002a; Sung and Chung, 2002], have focused primarily on extracellular matrix protein expression, growth factor secretion, angiogenesis stimulation, and altered cell proliferation and survival [Rowley et al., 1994; Gregoire and Lieubeau, 1995; Martin et al., 1996; Noel and Foidart, 1998; Rowley, 1998; Tuxhorn et al., 2001, 2002a,b]. Gap junctions between cells, and the restrictive, direct cytoplasmic transfer they allow, should not be overlooked as uniquely powerful players in the reciprocal cell–cell communication that accompanies cancer progression.

Gap junctions are formed of connexin molecules linked between opposing cells. Electron microscopy has revealed their existence in most tissues [Shivers and McVicar, 1995], including human bone [Cancelas et al., 2000; Durig et al., 2000; Tuxhorn et al., 2002a,b,c]. If cancer treatments are most effective when aimed at both stromal cells (soil) and cancerous prostate epithelial cells (seeds), then gap junctions between and among these cell compartments deserve special attention for therapy. Indeed, connexins have recently been found to be involved in the "bystander effect" in gene therapy experiments [Carystinos et al., 1999, 2001]. Gap junctional connections are not only important for normal cell growth regulation [White et al., 1995; Nicholson and Bruzzone, 1997], but connexin expression levels negatively correlate with cancer progression; that is, down-regulation of connexin proteins accompanies increased metastatic potential [Trosko and Ruch, 1998; Sulkowski et al., 1999; Carystinos et al., 2001; Naus, 2002; Trosko, 2003]. Levels of connexins 26, 32, and 43 are all known to decrease during cancer progression [Grossman et al., 1994; Tsuda et al., 1995; Gee et al., 2003], and some connexins have even been found to function as tumor suppressor genes [Cunha et al., 2002]. Furthermore, when certain connexins are overexpressed, a decrease in tumorgenicity has been observed [Hirschi et al., 1996; Mehta et al., 1999]. However, little is known about subtle cancerous changes in connexin usage, and even less about the direct mechanisms by which connexins affect the development of primary tumors or tumors at distant sites. Most metastases retain some connexin expression, raising the possibility that these proteins are involved in intercellular communication between cancer cells and the cells in their new habitat. Heterotypic gap junction formation is possible, both between connexins of different isotypes and between cell types [Bruzzone et al., 1996; Goodenough and Paul, 2003]. Heterotypic gap junctional communication does occur in both the bone marrow and hematopoetic tissues, and multiple connexins are known to be involved both during bone development and tissue homeostasis, in particular connexin 43 [Steinberg et al., 1994; Lecanda et al., 1998, Lecanda et al., 2000a,b]. In prostate cancer, several connexins have been analyzed, although with mixed results, as only a portion of the cells express connexins. Some researchers have detected connexins 32 and 40 in normal prostate, but not connexin 43 [Mehta et al., 1996; Kucuk et al., 2001]. Others have found decreased expression of connexins 32 and 43 in prostatic carcinoma [Tsai et al., 1996; Hossain et al., 1999a,b,c; Habermann et al., 2001], suggesting that they are still present in the metastatic tumors.

We are not aware of any studies correlating androgen levels, connexin expression, and tumor invasion and metastasis. Evidence that androgens regulate the expression of the connexin 43 gene in prostate tissue comes from studies in normal and castrated rats, where castration is associated with a dramatic and specific increase in connexin 43 mRNA and protein expression, and is correlated with increased apoptosis [Huynh et al., 2001]. Supplementing castrated animals with testosterone or DHT reverses this process. Thus, in castrated hosts, it is conceivable that connexins may actually play a role in increasing the ability of tumor cells to invade bone tissues, particularly through elevations of connexin 43 in men treated with androgen deprivation therapy.

CONCLUDING REMARKS

Recently, across cancer research fields, the interplay between cancer cells and their microenvironments has enjoyed increased attention, as reflected in the topic's designated status as an "extraordinary opportunity" by America's National Cancer Institute. Evidence is accumulating that prostate cancer depends especially strongly upon shifts in cell environment, or that acquired, rather than inherited, genetic alterations are key to its development and progression. At the time of disease diagnosis, prostate cancer is multifocal and heterogeneous,

with an average of five apparently independent cancer lesions and numerous other high-grade PIN lesions [Bastacky et al., 1995], and with each focus and lesion presenting separate genetic constitutions. Furthermore, the associations between prostate cancer development and diet, lifestyle, and/or levels of sex steroid are, in fact, stronger than those for sporadic and even familial forms of prostate cancer [Haenszel and Kurihara, 1968; Shimizu et al., 1991], although certain inherited genetic loci have been shown to contribute to familial prostate cancer [Ahlbom et al., 1997; Page et al., 1997; Lichtenstein et al., 2000].

Three future developments, having to do with tumor–microenvironment interactions, are particularly needed. First, improved methodologies for cell culture and in vivo molecular imaging will allow better recapitulation of normal development and cancer-stroma interplay, as well as imaging of proteins during cancer cell growth and locomotion. Second, the development of additional relevant cancer cell lines (including luminal and basal epithelial cell lines, as well as fibroblast, smooth muscle, myofibroblast, endothelial, and inflammatory cell lines), each representing different stages of prostate cancer development and progression, would provide invaluable tools for dissecting the features and requirements of invasive prostate cancer, and for differentiating between prostate cancers based on their degree of virulence. Third, molecular profiling of the signaling networks associated with soluble growth factors, insoluble extracellular matrices, and sex steroid hormones will aid in the identification, testing, and validation of selective anti-neoplastic agents.

Given the power of the microenvironment to direct prostate cancer progression, and the known preference of prostate cancer cells for the bone, we have reviewed here recent studies that address shifting prostate and bone stromal cell–cell and cell–matrix interactions during cancer progression. Increased knowledge of normal and aging bone stroma, reactive bone stroma, and the prostate cancer cell response to bone stroma, will ultimately reveal opportunities for improved therapy in the prevention and treatment of prostate cancer metastasis.

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REFERENCES

Ahlbom A, Lichtenstein P, Malmstrom H, Feychting M, Hemminki K, Pedersen NL. 1997. Cancer in twins: Genetic and nongenetic familial risk factors. J Natl Cancer Inst 89(4):287–293.

Akhurst RJ, Balmain A. 1999. Genetic events and the role of TGF beta in epithelial tumour progression. J Pathol 187(1):82–90.

Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ. 2000. Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: A new model for metastasis. Nat Med 6(1):100–102.

Allen MV, Smith GJ, Juliano R, Maygarden SJ, Mohler JL. 1998. Downregulation of the beta4 integrin subunit in prostatic carcinoma and prostatic intraepithelial neoplasia. Hum Pathol 29(4):311–318.

Andersen H, Jensen ON, Eriksen EF. 2003a. A proteome study of secreted prostatic factors affecting osteoblastic activity: Identification and characterisation of cyclophilin A. Eur J Cancer 39(7):989–995.

Andersen H, Jensen ON, Moiseeva EP, Eriksen EF. 2003b. A proteome study of secreted prostatic factors affecting osteoblastic activity: Galectin-1 is involved in differentiation of human bone marrow stromal cells. J Bone Miner Res 18(2):195–203.

Ankrom MA, Patterson JA, d'Avis PY, Vetter UK, Blackman MR, Sponseller PD, et al. 1998. Age-related changes in human oestrogen receptor alpha function and levels in osteoblasts. Biochem J 333(Pt 3):787–794.

Ashworth T. 1869. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Aust Med J 14:146–148.

Autzen P, Robson CN, Bjartell A, Malcolm AJ, Johnson MI, Neal DE, et al. 1998. Bone morphogenetic protein 6 in skeletal metastases from prostate cancer and other common human malignancies. Br J Cancer 78(9):1219–1223.

Bailey AJ, Paul RG, Knott L. 1998. Mechanisms of maturation and ageing of collagen. Mech Ageing Dev 106(1–2): 1–56.

Banerjee C, McCabe LR, Choi JY, Hiebert SW, Stein JL, Stein GS, et al. 1997. Runt homology domain proteins in osteoblast differentiation: AML3/CBFA1 is a major component of a bone-specific complex. J Cell Biochem 66(1): 1–8.

Basset P, Wolf C, Chambon P. 1993. Expression of the stromelysin-3 gene in fibroblastic cells of invasive carcinomas of the breast and other human tissues: A review. Breast Cancer Res Treat 24(3):185–193.

Bastacky SI, Wojno KJ, Walsh PC, Carmichael MJ, Epstein JI. 1995. Pathological features of hereditary prostate cancer. J Urol 153(3):987–992.

Bastón OV. 1940. The function of the vertebral veins and their role in the spread of metastases. Ann Surg 112: 138–149.

Bello-DeOcampo D, Kleinman HK, Webber MM. 2001. The role of alpha 6 beta 1 integrin and EGF in normal and malignant acinar morphogenesis of human prostatic epithelial cells. Mutat Res 480–481:209–217.

Billiard J, Moran RA, Whitley MZ, Chatterjee-Kishore M, Gillis K, Brown EL, et al. 2003. Transcriptional profiling of human osteoblast differentiation. J Cell Biochem 89(2): 389–400.

Bissell MJ, Radisky D. 2001. Putting tumours in context. Nat Rev Cancer 1(1):46–54.

Bissell MJ, Weaver VM, Lelievre SA, Wang F, Petersen OW, Schmeichel KL. 1999. Tissue structure, nuclear organization, and gene expression in normal and malignant breast. Cancer Res 59(7):1757–1763.

Bliss T, Errington M, Fransen E, Godfraind JM, Kauer JA, Kooy RF, et al. 2000. Long-term potentiation in mice lacking the neural cell adhesion molecule L1. Curr Biol 10(24):1607–1610.

Bonkhoff H, Stein U, Remberger K. 1993. Differential expression of alpha 6 and alpha 2 very late antigen integrins in the normal, hyperplastic, and neoplastic prostate: Simultaneous demonstration of cell surface receptors and their extracellular ligands. Hum Pathol 24(3):243–248.

Boonen S, Vanderschueren D, Cheng XG, Verbeke G, Dequeker J, Geusens P, et al. 1997. Age-related (type II) femoral neck osteoporosis in men: Biochemical evidence for both hypovitaminosis D- and androgen deficiency-induced bone resorption. J Bone Miner Res 12(12):2119–2126.

Boyce BF, Hughes DE, Wright KR, Xing L, Dai A. 1999. Recent advances in bone biology provide insight into the pathogenesis of bone diseases. Lab Invest 79(2):83–94.

Boyle WJ, Simonet WS, Lacey DL. 2003. Osteoclast differentiation and activation. Nature 423(6937):337–342.

Brekken RA, Sage EH. 2001. SPARC, a matricellular protein: At the crossroads of cell–matrix communication. Matrix Biol 19(8):816–827.

Brew K, Dinakarpandian D, Nagase H. 2000. Tissue inhibitors of metalloproteinases: Evolution, structure, and function. Biochim Biophys Acta 1477(1–2):267–283.

Brooks PC, Clark RA, Cheresh DA. 1994a. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 264(5158):569–571.

Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, et al. 1994b. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 79(7):1157–1164.

Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. 1995. Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. J Clin Invest 96(4):1815–1822.

Brown JM, Corey E, Lee ZD, True LD, Yun TJ, Tondravi M, et al. 2001a. Osteoprotegerin and rank ligand expression in prostate cancer. Urology 57(4):611–616.

Brown JM, Vessella RL, Kostenuik PJ, Dunstan CR, Lange PH, Corey E. 2001b. Serum osteoprotegerin levels are increased in patients with advanced prostate cancer. Clin Cancer Res 7(10):2977–2983.

Brubaker KD, Vessella RL, True LD, Thomas R, Corey E. 2003. Cathepsin K mRNA and protein expression in prostate cancer progression. J Bone Miner Res 18(2): 222–230.

Bruzzone R, White TW, Paul DL. 1996. Connections with connexins: The molecular basis of direct intercellular signaling. Eur J Biochem 238(1):1–27.

Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, et al. 2000. Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. Hum Pathol 31(5):578–583.

Calvo A, Xiao N, Kang J, Best CJ, Leiva I, Emmert-Buck MR, et al. 2002. Alterations in gene expression profiles during prostate cancer progression: Functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors. Cancer Res 62(18):5325–5335.

Cancelas JA, Koevoet WL, de Koning AE, Mayen AE, Rombouts EJ, Ploemacher RE. 2000. Connexin-43 gap junctions are involved in multi connexin-expressing stromal support of hemopoietic progenitors and stem cells. Blood 96(2):498–505.

Carystinos GD, Katabi MM, Laird DW, Galipeau J, Chan H, Alaoui-Jamali MA, et al. 1999. Cyclic-AMP induction of gap junctional intercellular communication increases bystander effect in suicide gene therapy. Clin Cancer Res 5(1):61–68.

Carystinos GD, Bier A, Batist G. 2001. The role of connexin-mediated cell–cell communication in breast cancer metastasis. J Mammary Gland Biol Neoplasia 6(4): 431–440.

Castro-Malaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, et al. 1980. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. Blood 56(2):289–301.

Chiao JW, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, et al. 2000. Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. Br J Cancer 83(3):360–365.

Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T. 1986. Tenascin: An extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47(1):131–139.

Chung LW. 1993. Implications of stromal–epithelial interaction in human prostate cancer growth, progression, and differentiation. Semin Cancer Biol 4(3):183–192.

Chung LW. 1995. The role of stromal–epithelial interaction in normal and malignant growth. Cancer Surveys 23: 33–42.

Cifuentes N, Pickren JW. 1979. Metastases from carcinoma of mammary gland: An autopsy study. J Surg Oncol 11(3):193–205.

Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, et al. 1998. Thrombospondin-1 is a major activator of TGF-beta1 in vivo. Cell 93(7): 1159–1170.

Cress AE, Rabinovitz I, Zhu W, Nagle RB. 1995. The alpha 6 beta 1 and alpha 6 beta 4 integrins in human prostate cancer progression. Cancer Metastasis Rev 14(3):219–228.

Cruveilhier LJB. 1829. Anatomie pathologique du corps humain, ou description, avec figures lithographiées et colorieés, des diverses alterations morbides dont le corps humain est susceptible. Paris: J. B. Baillière.

Cunha GR, Hayward SW, Wang YZ. 2002. Role of stroma in carcinogenesis of the prostate. Differentiation 70(9–10): 473–485.

D'Avis PY, Frazier CR, Shapiro JR, Fedarko NS. 1997. Age-related changes in effects of insulin-like growth factor I on human osteoblast-like cells. Biochem J 324(Pt 3):753–760.

Davis TL, Cress AE, Dalkin BL, Nagle RB. 2001. Unique expression pattern of the alpha6beta4 integrin and laminin-5 in human prostate carcinoma. Prostate 46(3): 240–248.

de la Piedra C, Castro-Errecaborde NA, Traba ML, Mendez-Davila C, Garcia-Moreno C, Rodriguez de Acuna L, et al. 2003. Bone remodeling markers in the detection

of bone metastases in prostate cancer. Clin Chim Acta 331(1–2):45–53.
De Wever O, Mareel M. 2003. Role of tissue stroma in cancer cell invasion. J Pathol 200(4):429–447.
Dedhar S, Saulnier R, Nagle R, Overall CM. 1993. Specific alterations in the expression of alpha 3 beta 1 and alpha 6 beta 4 integrins in highly invasive and metastatic variants of human prostate carcinoma cells selected by in vitro invasion through reconstituted basement membrane. Clin Exp Metastasis 11(5):391–400.
Dolo V, Ginestra A, Ghersi G, Nagase H, Vittorelli ML. 1994. Human breast carcinoma cells cultured in the presence of serum shed membrane vesicles rich in gelatinolytic activities. J Submicrosc Cytol Pathol 26(2):173–180.
Dolo V, Adobati E, Canevari S, Picone MA, Vittorelli ML. 1995. Membrane vesicles shed into the extracellular medium by human breast carcinoma cells carry tumor-associated surface antigens. Clin Exp Metastasis 13(4): 277–286.
Dolo V, Ginestra A, Cassara D, Violini S, Lucania G, Torrisi MR, et al. 1998. Selective localization of matrix metalloproteinase 9, beta1 integrins, and human lymphocyte antigen class I molecules on membrane vesicles shed by 8701-BC breast carcinoma cells. Cancer Res 58(19): 4468–4474.
Dolo V, D'Ascenzo S, Violini S, Pompucci L, Festuccia C, Ginestra A, et al. 1999. Matrix-degrading proteinases are shed in membrane vesicles by ovarian cancer cells in vivo and in vitro. Clin Exp Metastasis 17(2):131–140.
Dong Z, Nemeth JA, Cher ML, Palmer KC, Bright RC, Fridman R. 2001. Differential regulation of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 expression in co-cultures of prostate cancer and stromal cells. Int J Cancer 93(4):507–515.
Doucet C, Jasmin C, Azzarone B. 2000. Unusual interleukin-4 and -13 signaling in human normal and tumor lung fibroblasts. Oncogene 19(51):5898–5905.
Dougherty KM, Blomme EA, Koh AJ, Henderson JE, Pienta KJ, Rosol TJ, et al. 1999. Parathyroid hormone-related protein as a growth regulator of prostate carcinoma. Cancer Res 59(23):6015–6022.
Drake CJ, Cheresh DA, Little CD. 1995. An antagonist of integrin alpha v beta 3 prevents maturation of blood vessels during embryonic neovascularization. J Cell Sci 108(Pt 7):2655–2661.
Ducy P. 2000. Cbfa1: A molecular switch in osteoblast biology. Dev Dyn 219(4):461–471.
Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. 1997. Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. Cell 89(5):747–754.
Durig J, Rosenthal C, Halfmeyer K, Wiemann M, Novotny J, Bingmann D, et al. 2000. Intercellular communication between bone marrow stromal cells and CD34(+) haematopoietic progenitor cells is mediated by connexin 43-type gap junctions. Br J Haematol 111(2):416–425.
Edlund M, Miyamoto T, Sikes RA, Ogle R, Laurie GW, Farach-Carson MC, et al. 2001. Integrin expression and usage by prostate cancer cell lines on laminin substrata. Cell Growth Differ 12(2):99–107.
Eliceiri BP, Cheresh DA. 2000. Role of apha v integrins during angiogenesis. Cancer J 6(Suppl 3):S245–S249.
Eliceiri BP, Cheresh DA. 2001. Adhesion events in angiogenesis. Curr Opin Cell Biol 13(5):563–568.
Ellis WJ, Pfitzenmaier J, Colli J, Arfman E, Lange PH, Vessella RL. 2003. Detection and isolation of prostate cancer cells from peripheral blood and bone marrow. Urology 61(2):277–281.
Engell HC. 1955. Cancer cells in the circulating blood. Acta Chir Scand Suppl 201:1–70.
Fedarko NS, Termine JD, Young MF, Robey PG. 1990. Temporal regulation of hyaluronan and proteoglycan metabolism by human bone cells in vitro. J Biol Chem 265(21):12200–12209.
Fedarko NS, Vetter UK, Weinstein S, Robey PG. 1992. Age-related changes in hyaluronan, proteoglycan, collagen, and osteonectin synthesis by human bone cells. J Cell Physiol 151(2):215–227.
Fedarko NS, Vetter UK, Robey PG. 1995. Age-related changes in bone matrix structure in vitro. Calcif Tissue Int 56(Suppl 1):S41–S43.
Fidler IjaN, G L. 1976. Organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. J Natl Cancer Inst 57:1199–1202.
Fizazi K, Yang J, Peleg S, Sikes CR, Kreimann EL, Daliani D, et al. 2003. Prostate cancer cells–osteoblast interaction shifts expression of growth/survival-related genes in prostate cancer and reduces expression of osteoprotegerin in osteoblasts. Clin Cancer Res 9(7):2587–2597.
Fornaro M, Tallini G, Bofetiado CJ, Bosari S, Languino LR. 1996. Down-regulation of beta 1C integrin, an inhibitor of cell proliferation, in prostate carcinoma. Am J Pathol 149(3):765–773.
Fornaro M, Tallini G, Zheng DQ, Flanagan WM, Manzotti M, Languino LR. 1999. p27(kip1) acts as a downstream effector of and is coexpressed with the beta1C integrin in prostatic adenocarcinoma. J Clin Invest 103(3):321–329.
Fransen E, Van Camp G, Vits L, Willems PJ. 1997. L1-associated diseases: Clinical geneticists divide, molecular geneticists unite. Hum Mol Genet 6(10):1625–1632.
Fransen E, D'Hooge R, Van Camp G, Verhoye M, Sijbers J, Reyniers E, et al. 1998. L1 knockout mice show dilated ventricles, vermis hypoplasia, and impaired exploration patterns. Hum Mol Genet 7(6):999–1009.
Freedland SJ, Seligson DB, Liu AY, Pantuck AJ, Paik SH, Horvath S, et al. 2003. Loss of CD10 (neutral endopeptidase) is a frequent and early event in human prostate cancer. Prostate 55(1):71–80.
Gabrielsen TO, Brandtzaeg P, Hoel PS, Dale I. 1988. Epithelial distribution of a myelomonocytic antigen LI in relation to cutaneous malignancies and melanocytic naevi. Br J Dermatol 118(1):59–67.
Garcia-Moreno C, Mendez-Davila C, de La Piedra C, Castro-Errecaborde NA, Traba ML. 2002. Human prostatic carcinoma cells produce an increase in the synthesis of interleukin-6 by human osteoblasts. Prostate 50(4): 241–246.
Gay S, Gay RE, Prchal JT. 1984. Immunohistological studies of bone marrow collagen. Prog Clin Biol Res 154: 291–306.
Gee J, Tanaka M, Grossman HB. 2003. Connexin 26 is abnormally expressed in bladder cancer. J Urol 169(3): 1135–1137.
Gimble JM, Wanker F, Wang CS, Bass H, Wu X, Kelly K, et al. 1994. Regulation of bone marrow stromal cell differentiation by cytokines whose receptors share the gp130 protein. J Cell Biochem 54(1):122–133.

Ginestra A, La Placa MD, Saladino F, Cassara D, Nagase H, Vittorelli ML. 1998. The amount and proteolytic content of vesicles shed by human cancer cell lines correlates with their in vitro invasiveness. Anticancer Res 18(5A): 3433–3437.

Ginestra A, Miceli D, Dolo V, Romano FM, Vittorelli ML. 1999. Membrane vesicles in ovarian cancer fluids: A new potential marker. Anticancer Res 19(4C):3439–3445.

Gleave M, Hsieh JT, Gao CA, von Eschenbach AC, Chung LW. 1991. Acceleration of human prostate cancer growth in vivo by factors produced by prostate and bone fibroblasts. Cancer Res 51(14):3753–3761.

Glinsky GV, Glinsky VV, et al. 1996. Apoptosis amd metastasis: A superior resistance of metastatic cancer cells to programmed cell death. Cancer Lett 101(1): 43–51.

Glinsky VV, Glinsky GV, Glinskii OV, Huxley VH, Turk JR, Mossine VV, et al. 2003. Intravascular metastatic cancer cell homotypic aggregation at the sites of primary attachment to the endothelium. Cancer Res 63(13):3805–3811.

Gohji K, Fujimoto N, Hara I, Fujii A, Gotoh A, Okada H, et al. 1998. Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. Int J Cancer 79(1):96–101.

Goodenough DA, Paul DL. 2003. Beyond the gap: Functions of unpaired connexon channels. Nat Rev Mol Cell Biol 4(4):285–294.

Gregoire M, Lieubeau B. 1995. The role of fibroblasts in tumor behavior. Cancer Metastasis Rev 14(4):339–350.

Grossman HB, Liebert M, Lee IW, Lee SW. 1994. Decreased connexin expression and intercellular communication in human bladder-cancer cells. Cancer Res 54(11):3062–3065.

Guise TA, Yin JJ, Mohammad KS. 2003. Role of endothelin-1 in osteoblastic bone metastases. Cancer 97(Suppl 3): 779–784.

Gutheil JC, Campbell TN, Pierce PR, Watkins JD, Huse WD, Bodkin DJ, et al. 2000. Targeted antiangiogenic therapy for cancer using vitaxin: A humanized monoclonal antibody to the integrin alphavbeta3. Clin Cancer Res 6(8):3056–3061.

Gutwein P, Oleszewski M, Mechtersheimer S, Agmon-Levin N, Krauss K, Altevogt P. 2000. Role of Src kinases in the ADAM-mediated release of L1 adhesion molecule from human tumor cells. J Biol Chem 275(20):15490–15497.

Habermann H, Ray V, Habermann W, Prins GS. 2001. Alterations in gap junction protein expression in human benign prostatic hyperplasia and prostate cancer. J Urol 166(6):2267–2272.

Hadley JC, Meek KM, Malik NS. 1998. Glycation changes the charge distribution of type I collagen fibrils. Glycoconj J 15(8):835–840.

Haenszel W, Kurihara M. 1968. Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. J Natl Cancer Inst 40(1):43–68.

Haier J, Nicolson GL. 2001a. The role of tumor cell adhesion as an important factor in formation of distant colorectal metastasis. Dis Colon Rectum 44(6):876–884.

Haier J, Nicolson GL. 2001b. Tumor cell adhesion under hydrodynamic conditions of fluid flow. Apmis 109(4): 241–262.

Hao J, Jackson L, Calaluce R, McDaniel K, Dalkin BL, Nagle RB. 2001. Investigation into the mechanism of the loss of laminin 5 (alpha3beta3gamma2) expression in prostate cancer. Am J Pathol 158(3):1129–1135.

Haq M, Goltzman D, Tremblay G, Brodt P. 1992. Rat prostate adenocarcinoma cells disseminate to bone and adhere preferentially to bone marrow-derived endothelial cells. Cancer Res 52(17):4613–46119.

Hart IR. 1982. 'Seed and soil' revisited: Mechanisms of site-specific metastasis. Cancer Metastasis Rev 1(1):5–16.

Hartstein ME, Grove ASJ, Woog JJ. 1997. The role of the integrin family of adhesion molecules in the development of tumors metastatic to the orbit. Ophthal Plast Reconstr Surg 13(4):227–238.

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. 2003. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374(Pt 1):1–20.

Hermann M, Untergasser G, Rumpold H, Berger P, et al. 2000. Aging of the male reproductive system. Exp Gerontol 35(9–10):1267–1279.

Hill PA, Murphy G, Docherty AJ, Hembry RM, Millican TA, Reynolds JJ, et al. 1994. The effects of selective inhibitors of matrix metalloproteinases (MMPs) on bone resorption and the identification of MMPs and TIMP-1 in isolated osteoclasts. J Cell Sci 107(Pt 11):3055–3064.

Hill PA, Docherty AJ, Bottomley KM, O'Connell JP, Morphy JR, Reynolds JJ, et al. 1995. Inhibition of bone resorption in vitro by selective inhibitors of gelatinase and collagenase. Biochem J 308(Pt 1):167–175.

Hirschi KK, Xu CE, Tsukamoto T, Sager R. 1996. Gap junction genes *Cx26* and *Cx43* individually suppress the cancer phenotype of human mammary carcinoma cells and restore differentiation potential. Cell Growth Differ 7(7):861–870.

Hoffman KB. 1998. The relationship between adhesion molecules and neuronal plasticity. Cell Mol Neurobiol 18(5):461–475.

Hofmann UB, Westphal JR, Van Kraats AA, Ruiter DJ, Van Muijen GN. 2000. Expression of integrin alpha(v)-beta(3) correlates with activation of membrane-type matrix metalloproteinase-1 (MT1-MMP) and matrix metalloproteinase-2 (MMP-2) in human melanoma cells in vitro and in vivo. Int J Cancer 87(1):12–19.

Holbro T, Civenni G, Hynes NE. 2003. The ErbB receptors and their role in cancer progression. Exp Cell Res 284(1): 99–110.

Hossain MZ, Jagdale AB, Ao P, Boynton AL. 1999a. Mitogen-activated protein kinase and phosphorylation of connexin43 are not sufficient for the disruption of gap junctional communication by platelet-derived growth factor and tetradecanoylphorbol acetate. J Cell Physiol 179(1):87–96.

Hossain MZ, Jagdale AB, Ao P, Kazlauskas A, Boynton AL. 1999b. Disruption of gap junctional communication by the platelet-derived growth factor is mediated via multiple signaling pathways. J Biol Chem 274(15):10489–10496.

Hossain MZ, Jagdale AB, Ao P, LeCiel C, Huang RP, Boynton AL. 1999c. Impaired expression and posttranslational processing of connexin43 and downregulation of gap junctional communication in neoplastic human prostate cells. Prostate 38(1):55–59.

Huynh HT, Alpert L, Laird DW, Batist G, Chalifour L, Alaoui-Jamali MA. 2001. Regulation of the gap junction connexin 43 gene by androgens in the prostate. J Mol Endocrinol 26(1):1–10.

Iannotti JP. 1990. Growth plate physiology and pathology. Orthop Clin N Am 21(1):1–17.

Inoue S, Osmond DG. 2001. Basement membrane of mouse bone marrow sinusoids shows distinctive structure and proteoglycan composition: A high resolution ultrastructural study. Anat Rec 264(3):294–304.

Jaffe EA, Minick CR, Adelman B, Becker CG, Nachman R. 1976. Synthesis of basement membrane collagen by cultured human endothelial cells. J Exp Med 144(1): 209–225.

Jay PR, Centrella M, Lorenzo J, Bruce AG, Horowitz MC. 1996. Oncostatin-M: A new bone active cytokine that activates osteoblasts and inhibits bone resorption. Endocrinology 137(4):1151–1158.

Juliano RL, Varner JA. 1993. Adhesion molecules in cancer: The role of integrins. Curr Opin Cell Biol 5(5):812–818.

Kadmon G, Altevogt P. 1997. The cell adhesion molecule L1: Species- and cell-type-dependent multiple binding mechanisms. Differentiation 61(3):143–150.

Karsenty G. 2000. Role of Cbfa1 in osteoblast differentiation and function. Semin Cell Dev Biol 11(5):343–346.

Keller ET. 2002. The role of osteoclastic activity in prostate cancer skeletal metastases. Drugs Today (Barc) 38(2): 91–102.

Kelm RJ, Jr., Hair GA, Mann KG, Grant BW. 1992. Characterization of human osteoblast and megakaryocyte-derived osteonectin (SPARC). Blood 80(12):3112–3119.

Kim CW, Lee HM, Lee TH, Kang C, Kleinman HK, Gho YS. 2002. Extracellular membrane vesicles from tumor cells promote angiogenesis via sphingomyelin. Cancer Res 62(21):6312–6317.

Klein G. 1995. The extracellular matrix of the hematopoietic microenvironment. Experientia 51(9–10):914–926.

Knox JD, Cress AE, Clark V, Manriquez L, Affinito KS, Dalkin BL, et al. 1994. Differential expression of extracellular matrix molecules and the alpha 6-integrins in the normal and neoplastic prostate. Am J Pathol 145(1):167–174.

Koeneman KS, Kao C, Ko SC, Yang L, Wada Y, Kallmes DF, et al. 2000. Osteocalcin-directed gene therapy for prostate-cancer bone metastasis. World J Urol 18(2):102–110.

Kucuk O, Sarkar FH, Sakr W, Djuric Z, Pollak MN, Khachik F, et al. 2001. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiol Biomarkers Prev 10(8):861–868.

Kujat R, Miragall F, Krause D, Dermietzel R, Wrobel KH. 1995. Immunolocalization of the neural cell adhesion molecule L1 in non-proliferating epithelial cells of the male urogenital tract. Histochem Cell Biol 103(4):311–321.

Kumar CC. 2003. Integrin alpha v beta 3 as a therapeutic target for blocking tumor-induced angiogenesis. Curr Drug Targets 4(2):123–131.

Kumar CC, Malkowski M, Yin Z, Tanghetti E, Yaremko B, Nechuta T, et al. 2001. Inhibition of angiogenesis and tumor growth by SCH221153, a dual alpha(v)beta3 and alpha(v)beta5 integrin receptor antagonist. Cancer Res 61(5):2232–2238.

Lang SH, Miller WR, Habib FK. 1995. Stimulation of human prostate cancer cell lines by factors present in human osteoblast-like cells but not in bone marrow. Prostate 27(5):287–293.

Lebovitz HE, Eisenbarth GS. 1975. Hormonal regulation of cartilage growth and metabolism. Vitam Horm 33:575–648.

Lecanda F, Towler DA, Ziambaras K, Cheng SL, Koval M, Steinberg TH, et al. 1998. Gap junctional communication modulates gene expression in osteoblastic cells. Mol Biol Cell 9(8):2249–2258.

Lecanda F, Warlow PM, Sheikh S, Furlan F, Steinberg TH, Civitelli R. 2000a. Connexin43 deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. J Cell Biol 151(4):931–943.

Lecanda F, Warlow PM, Sheikh S, Furlan F, Steinberg TH, Civitelli R. 2000b. Connexin43 deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. J Cell Biol 151(4):931–944.

Lee Y, Schwarz E, Davies M, Jo M, Gates J, Wu J, et al. 2003. Differences in the cytokine profiles associated with prostate cancer cell induced osteoblastic and osteolytic lesions in bone. J Orthop Res 21(1):62–72.

Lehr JE, Pienta KJ. 1998. Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. J Natl Cancer Inst 90(2):118–123.

Lerner MP, Lucid SW, Wen GJ, Nordquist RE. 1983. Selected area membrane shedding by tumor cells. Cancer Lett 20(2):125–130.

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. 2000. Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343(2):78–85.

Lin DL, Tarnowski CP, Zhang J, Dai J, Rohn E, Patel AH, et al. 2001. Bone metastatic LNCaP-derivative C4-2B prostate cancer cell line mineralizes in vitro. Prostate 47(3):212–221.

Linnemann D, Raz A, Bock E. 1989. Differential expression of cell adhesion molecules in variants of K1735 melanoma cells differing in metastatic capacity. Int J Cancer 43(4):709–712.

Liotta LA, Kohn EC. 2001. The microenvironment of the tumour–host interface. Nature 411(6835):375–379.

Liotta LA, Saidel MG, Kleinerman J. 1976. The significance of hematogenous tumor cell clumps in the metastatic process. Cancer Res 36(3):889–894.

Liotta LA, Steeg PS, Stetler-Stevenson WG. 1991. Cancer metastasis and angiogenesis: An imbalance of positive and negative regulation. Cell 64(2):327–336.

Lohr M, Schmidt C, Ringel J, Kluth M, Muller P, Nizze H, et al. 2001. Transforming growth factor-beta1 induces desmoplasia in an experimental model of human pancreatic carcinoma. Cancer Res 61(2):550–555.

Lyons RM, Gentry LE, Purchio AF, Moses HL. 1990. Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. J Cell Biol 110(4):1361–1367.

Martin M, Pujuguet P, Martin F. 1996. Role of stromal myofibroblasts infiltrating colon cancer in tumor invasion. Pathol Res Pract 192(7):712–717.

McEver RP. 1997. Selectin–carbohydrate interactions during inflammation and metastasis. Glycoconj J 14(5): 585–591.

Mehta PP, Lokeshwar BL, Schiller PC, Bendix MV, Ostenson RC, Howard GA, et al. 1996. Gap-junctional

communication in normal and neoplastic prostate epithelial cells and its regulation by cAMP. Mol Carcinog 15(1): 18–32.

Mehta PP, Perez-Stable C, Nadji M, Mian M, Asotra K, Roos BA. 1999. Suppression of human prostate cancer cell growth by forced expression of connexin genes. Dev Genet 24(1–2):91–110.

Mira E, Lacalle RA, Gonzalez MA, Gomez-Mouton C, Abad JL, Bernad A, et al. 2001. A role for chemokine receptor transactivation in growth factor signaling. EMBO Rep 2(2):151–156.

Miura S, Kodaira S, Hosoda Y. 1993. Immunohistologic analysis of the extracellular matrix components of the fibrous stroma of human colon cancer. J Surg Oncol 53(1): 36–42.

Miyata T, Notoya K, Yoshida K, Horie K, Maeda K, Kurokawa K, et al. 1997. Advanced glycation end products enhance osteoclast-induced bone resorption in cultured mouse unfractionated bone cells and in rats implanted subcutaneously with devitalized bone particles. J Am Soc Nephrol 8(2):260–270.

Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. 2000. Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: Implications for tumorigenesis. Cancer Res 60(9):2562–2566.

Moses MA, Wiederschain D, Loughlin KR, Zurakowski D, Lamb CC, Freeman MR. 1998. Increased incidence of matrix metalloproteinases in urine of cancer patients. Cancer Res 58(7):1395–1399.

Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, et al. 2002. The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. J Cell Biol 157(3): 493–507.

Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410(6824):50–56.

Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, et al. 1999. The integrin alpha v beta 6 binds and activates latent TGF beta 1: A mechanism for regulating pulmonary inflammation and fibrosis. Cell 96(3): 319–328.

Murant SJ, Handley J, Stower M, Reid N, Cussenot O, Maitland NJ. 1997. Co-ordinated changes in expression of cell adhesion molecules in prostate cancer. Eur J Cancer 33(2):263–271.

Nagakawa O, Murakami K, Yamaura T, Fujiuchi Y, Murata J, Fuse H, et al. 2000. Expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) on prostate cancer cell lines. Cancer Lett 155(2):173–179.

Nagase H. 1997. Activation mechanisms of matrix metalloproteinases. Biol Chem 378(3–4):151–160.

Nagle RB, Knox JD, Wolf C, Bowden GT, Cress AE. 1994. Adhesion molecules, extracellular matrix, and proteases in prostate carcinoma. J Cell Biochem Suppl 19:232–237.

Nagle RB, Hao J, Knox JD, Dalkin BL, Clark V, Cress AE. 1995. Expression of hemidesmosomal and extracellular matrix proteins by normal and malignant human prostate tissue. Am J Pathol 146(6):1498–1507.

Naus CC. 2002. Gap junctions and tumour progression. Can J Physiol Pharmacol 80(2):136–141.

Neaud V, Dubuisson L, Balabaud C, Bioulac-Sage P. 1995. Ultrastructure of human Kupffer cells maintained in culture. J Submicrosc Cytol Pathol 27(2):161–170.

Nemeth JA, Yousif R, Herzog M, Che M, Upadhyay J, Shekarriz B, et al. 2002. Matrix metalloproteinase activity, bone matrix turnover, and tumor cell proliferation in prostate cancer bone metastasis. J Natl Cancer Inst 94(1):17–25.

Nicholson SM, Bruzzone R. 1997. Gap junctions: Getting the message through. Curr Biol 7(6):R340–R344.

Noel A, Foidart JM. 1998. The role of stroma in breast carcinoma growth in vivo. J Mammary Gland Biol Neoplasia 3(2):215–225.

Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. 1997. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem 272(4):2446–2451.

Orlandini M, Oliviero S. 2001. In fibroblasts Vegf-D expression is induced by cell–cell contact mediated by cadherin-11. J Biol Chem 276(9):6576–6581.

Page WF, Braun MM, Partin AW, Caporaso N, Walsh P. 1997. Heredity and prostate cancer: A study of World War II veteran twins. Prostate 33(4):240–245.

Pancook JD, Reisfeld RA, Varki N, Vitiello A, Fox RI, Montgomery AM. 1997. Expression and regulation of the neural cell adhesion molecule L1 on human cells of myelomonocytic and lymphoid origin. J Immunol 158(9): 4413–4421.

Pasco S, Han J, Gillery P, Bellon G, Maquart FX, Borel JP, et al. 2000. A specific sequence of the noncollagenous domain of the alpha3(IV) chain of type IV collagen inhibits expression and activation of matrix metalloproteinases by tumor cells. Cancer Res 60(2):467–473.

Perlino E, Lovecchio M, Vacca RA, Fornaro M, Moro L, Ditonno P, et al. 2000. Regulation of mRNA and protein levels of beta1 integrin variants in human prostate carcinoma. Am J Pathol 157(5):1727–1734.

Petitclerc E, Boutaud A, Prestayko A, Xu J, Sado Y, Ninomiya Y, et al. 2000. New functions for non-collagenous domains of human collagen type IV. Novel integrin ligands inhibiting angiogenesis and tumor growth in vivo. J Biol Chem 275(11):8051–8061.

Pfeilschifter J, Diel I, Pilz U, Brunotte K, Naumann A, Ziegler R. 1993. Mitogenic responsiveness of human bone cells in vitro to hormones and growth factors decreases with age. J Bone Miner Res 8(6):707–717.

Pfeilschifter J, Diel I, Kloppinger T, Bismar H, Schuster EM, Balbach S, et al. 2000. Concentrations of insulin-like growth factor (IGF)-I, -II, and IGF binding protein-4, and -5 in human bone cell conditioned medium do not change with age. Mech Ageing Dev 117(1–3):109–114.

Pfitzenmaier J, Vessella R, Higano CS, Noteboom JL, Wallace D, Jr., Corey E. 2003. Elevation of cytokine levels in cachectic patients with prostate carcinoma. Cancer 97(5):1211–1216.

Quarles LD, Yohay DA, Lever LW, Caton R, Wenstrup RJ. 1992. Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: An in vitro model of osteoblast development. J Bone Miner Res 7(6):683–692.

Rabinovitz I, Nagle RB, Cress AE. 1995. Integrin alpha 6 expression in human prostate carcinoma cells is associated with a migratory and invasive phenotype in vitro and in vivo. Clin Exp Metastasis 13(6):481–491.

Reddi AH, Gay R, Gay S, Miller EJ. 1977. Transitions in collagen types during matrix-induced cartilage, bone, and bone marrow formation. Proc Natl Acad Sci USA 74(12):5589–5592.

Redler P, Lustig ES. 1968. Differences in the growth-promoting effect of normal and peritumoral dermis on epidermis in vitro. Dev Biol 17(6):679–691.

Redler P, Lustig ES. 1970. Control of epithelial development in normal and pathological connective tissue from oral mucosa. Dev Biol 22(1):84–95.

Reid RA, Hemperly JJ. 1992. Variants of human L1 cell adhesion molecule arise through alternate splicing of RNA. J Mol Neurosci 3(3):127–135.

Resnick MI. 1992. Hemodynamics of prostate cancer bone metastases. Adv Exp Med Biol 324:77–81.

Reynolds LE, Wyder L, Lively JC, Taverna D, Robinson SD, Huang X, et al. 2002. Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. Nat Med 8(1):27–34.

Rhee HW, Zhau HE, Pathak S, Multani AS, Pennanen S, Visakorpi T, et al. 2001. Permanent phenotypic and genotypic changes of prostate cancer cells cultured in a three-dimensional rotating-wall vessel. In Vitro Cell Dev Biol Anim 37(3):127–140.

Roberts S, Watne A, McGrath R, McGrew E, Cole WH. 1958. Technique and results of isolation of cancer cells from the circulating blood. AMA Arch Surg 76: 334–346.

Rodan GA, Noda M. 1991. Gene expression in osteoblastic cells. Crit Rev Eukaryot Gene Expr 1(2):85–98.

Ronnov-Jessen L, Petersen OW, Koteliansky VE, Bissell MJ. 1995. The origin of the myofibroblasts in breast cancer. Recapitulation of tumor environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells. J Clin Invest 95(2): 859–873.

Ronnov-Jessen L, Petersen OW, Bissell MJ. 1996. Cellular changes involved in conversion of normal to malignant breast: Importance of the stromal reaction. Physiol Rev 76(1):69–125.

Roodman GD. 2003. Role of stromal-derived cytokines and growth factors in bone metastasis. Cancer 97(Suppl 3): 733–738.

Rosen CJ. 2000. Growth hormone and aging. Endocrine 12(2):197–201.

Rowley DR. 1998. What might a stromal response mean to prostate cancer progression? Cancer Metastasis Rev 17(4):411–419.

Rowley DR, Gerdes MJ, Dang TD, Mcbride L. 1994. Prostate stroma-specific gene-expression. J Cell Biochem 244–244.

Saiki I, Naito S, Yoneda J, Azuma I, Price JE, Fidler IJ. 1991. Characterization of the invasive and metastatic phenotype in human renal cell carcinoma. Clin Exp Metastasis 9(6):551–566.

Sanchez KM, Sweeney CJ, Mass R, Koch MO, Eckert GJ, Geary WA, et al. 2002. Evaluation of HER-2/neu expression in prostatic adenocarcinoma: A requested for a standardized, organ specific methodology. Cancer 95(8):1650–1655.

Schnitzer JE, McKinstry P, Light TR, Ogden JA. 1982. Quantitation of regional chondro-osseous circulation in canine tibia and femur. Am J Physiol 242(3):H365–H375.

Scott LJ, Clarke NW, George NJ, Shanks JH, Testa NG, Lang SH. 2001. Interactions of human prostatic epithelial cells with bone marrow endothelium: Binding and invasion. Br J Cancer 84(10):1417–1423.

Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. 1991. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 63(6):963–966.

Shivers R, McVicar L. 1995. Gap junctions revealed by freezefracture electron microscopy. Microscope Res Tech 31:437–445.

Siler U, Seiffert M, Puch S, Richards A, Torok-Storb B, Muller CA, et al. 2000. Characterization and functional analysis of laminin isoforms in human bone marrow. Blood 96(13):4194–4203.

Siler U, Rousselle P, Muller CA, Klein G. 2002. Laminin gamma2 chain as a stromal cell marker of the human bone marrow microenvironment. Br J Haematol 119(1): 212–220.

Singer C, Rasmussen A, Smith HS, Lippman ME, Lynch HT, Cullen KJ. 1995. Malignant breast epithelium selects for insulin-like growth factor II expression in breast stroma: Evidence for paracrine function. Cancer Res 55(11):2448–2454.

Sokoloff MH, Tso CL, Kaboo R, Taneja S, Pang S, deKernion JB, et al. 1996. In vitro modulation of tumor progression-associated properties of hormone refractory prostate carcinoma cell lines by cytokines. Cancer 77(9): 1862–1872.

Steinberg TH, Civitelli R, Geist ST, Robertson AJ, Hick E, Veenstra RD, et al. 1994. Connexin43 and connexin45 form gap junctions with different molecular permeabilities in osteoblastic cells. Embo J 13(4):744–750.

Sulkowski S, Sulkowska M, Skrzydlewska E. 1999. Gap junctional intercellular communication and carcinogenesis. Pol J Pathol 50(4):227–233.

Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, et al. 2003. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. J Cell Biochem 89(3):462–473.

Sung SY, Chung LW. 2002. Prostate tumor–stroma interaction: Molecular mechanisms and opportunities for therapeutic targeting. Differentiation 70(9–10):506–521.

Svennevig JL. 1980. In situ identification of inflammatory cells in malignant, non-lymphoid human tumours. Acta Pathol Microbiol Scand [A] 88(6):387–395.

Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. 2002. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 62(6):1832–1837.

Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. 1995. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. J Biol Chem 270(9):4689–4696.

ten Dijke P, Heldin CH. 1999. Signal transduction. An anchor for activation. Nature 397(6715):109–111.

Termine JD. 1990. Cellular activity, matrix proteins, and aging bone. Exp Gerontol 25(3–4):217–221.

Thalmann GN, Anezinis PE, Chang SM, Zhau HE, Kim EE, Hopwood VL. 1994. Androgen-independent cancer progression and bone metastasis in the LNCaP model of

human prostate cancer [erratum appears in Cancer Res 1994 Jul 15;54(14):3953]. Cancer Res 54(10):2577–2581.

Thalmann GN, Sikes RA, Wu TT, Degeorges A, Chang SM, Ozen M, et al. 2000. LNCaP progression model of human prostate cancer: Androgen-independence and osseous metastasis. Prostate 44(2):91–103. Jul 1;44(2).

Thelen K, Kedar V, Panicker AK, Schmid RS, Midkiff BR, Maness PF. 2002. The neural cell adhesion molecule L1 potentiates integrin-dependent cell migration to extracellular matrix proteins. J Neurosci 22(12):4918–4931.

Thomas R, True LD, Bassuk JA, Lange PH, Vessella RL. 2000. Differential expression of osteonectin/SPARC during human prostate cancer progression. Clin Cancer Res 6(3):1140–1149.

Thomas R, True LD, Lange PH, Vessella RL. 2001. Placental bone morphogenetic protein (*PLAB*) gene expression in normal, pre-malignant, and malignant human prostate: Relation to tumor development and progression. Int J Cancer 93(1):47–52.

Tintut Y, Parhami F, Le V, Karsenty G, Demer LL. 1999. Inhibition of osteoblast-specific transcription factor Cbfa1 by the cAMP pathway in osteoblastic cells. Ubiquitin/proteasome-dependent regulation. J Biol Chem 274(41):28875–28879.

Trosko JE. 2003. The role of stem cells and gap junctional intercellular communication in carcinogenesis. J Biochem Mol Biol 36(1):43–48.

Trosko JE, Ruch RJ. 1998. Cell–cell communication in carcinogenesis. Front Biosci 3:D208–D236.

Ts'o PO, Pannek J, Wang ZP, Lesko SA, Bova GS, Partin AW. 1997. Detection of intact prostate cancer cells in the blood of men with prostate cancer. Urology 49(6):881–885.

Tsai H, Werber J, Davia MO, Edelman M, Tanaka KE, Melman A, et al. 1996. Reduced connexin 43 expression in high grade, human prostatic adenocarcinoma cells. Biochem Biophys Res Commun 227(1):64–69.

Tsingotjidou AS, Zotalis G, Jackson KR, Sawyers C, Puzas JE, Hicks DG, et al. 2001. Development of an animal model for prostate cancer cell metastasis to adult human bone. Anticancer Res 21(2A):971–978.

Tsuda H, Asamoto M, BabaToriyama H, Iwahori Y, Hori T, Kim DJ, et al. 1995. Clofibrate-induced neoplastic development in the rat liver is associated with decreased connexin 32 expression but not with a co-ordinated shift in expression of marker enzymes. Toxicol Lett 82(3):693–699.

Tuxhorn JA, Ayala GE, Rowley DR. 2001. Reactive stroma in prostate cancer progression. J Urol 166(6):2472–2483.

Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. 2002a. Reactive stroma in human prostate cancer: Induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res 8(9):2912–2923.

Tuxhorn JA, McAlhany SJ, Dang TD, Ayala GE, Rowley DR. 2002b. Stromal cells promote angiogenesis and growth of human prostate tumors in a differential reactive stroma (DRS) xenograft model. Cancer Res 62(11):3298–+.

Tuxhorn JA, McAlhany SJ, Yang F, Dang TD, Rowley DR. 2002c. Inhibition of transforming growth factor-beta activity decreases angiogenesis in a human prostate cancer-reactive stroma xenograft model. Cancer Res 62(21):6021–6025.

Udayakumar TS, Chen ML, Bair EL, Von Bredow DC, Cress AE, Nagle RB, et al. 2003. Membrane type-1-matrix metalloproteinase expressed by prostate carcinoma cells cleaves human laminin-5 beta3 chain and induces cell migration. Cancer Res 63(9):2292–2299.

Upadhyay J, Shekarriz B, Nemeth JA, Dong Z, Cummings GD, Fridman R, et al. 1999. Membrane type 1-matrix metalloproteinase (MT1-MMP) and MMP-2 immunolocalization in human prostate: Change in cellular localization associated with high-grade prostatic intraepithelial neoplasia. Clin Cancer Res 5(12):4105–4110.

van Blitterswijk WJ, Emmelot P, Hilkmann HA, Hilgers J, Feltkamp CA. 1979. Rigid plasma-membrane-derived vesicles, enriched in tumour-associated surface antigens (MLr), occurring in the ascites fluid of a murine leukaemia (GRSL). Int J Cancer 23(1):62–70.

van Blitterswijk WJ, De Veer G, Krol JH, Emmelot P. 1982. Comparative lipid analysis of purified plasma membranes and shed extracellular membrane vesicles from normal murine thymocytes and leukemic GRSL cells. Biochim Biophys Acta 688(2):495–504.

van der Flier A, Sonnenberg A. 2001. Function and interactions of integrins. Cell Tissue Res 305(3):285–298.

Waltregny D, Bellahcene A, de Leval X, Florkin B, Weidle U, Castronovo V. 2000. Increased expression of bone sialoprotein in bone metastases compared with visceral metastases in human breast and prostate cancers. J Bone Miner Res 15(5):834–843.

Wang ZP, Eisenberger MA, Carducci MA, Partin AW, Scher HI, Ts'o PO. 2000. Identification and characterization of circulating prostate carcinoma cells. Cancer 88(12):2787–2795.

Wang J, Torbenson M, Wang Q, Ro JY, Becich M. 2003. Expression of inducible nitric oxide synthase in paired neoplastic and non-neoplastic primary prostate cell cultures and prostatectomy specimen. Urol Oncol 21(2):117–122.

White TW, Bruzzone R, Paul DL. 1995. The connexin family of intercellular channel-forming proteins. Kidney Int 48(4):1148–1157.

Wood M, Fudge K, Mohler JL, Frost AR, Garcia F, Wang M, et al. 1997. In situ hybridization studies of metalloproteinases 2 and 9 and TIMP-1 and TIMP-2 expression in human prostate cancer. Clin Exp Metastasis 15(3):246–258.

Wright JH, McDonnell S, Portella G, Bowden GT, Balmain A, Matrisian LM. 1994. A switch from stromal to tumor cell expression of stromelysin-1 mRNA associated with the conversion of squamous to spindle carcinomas during mouse skin tumor progression. Mol Carcinog 10(4):207–215.

Xiao ZS, Thomas R, Hinson TK, Quarles LD. 1998. Genomic structure and isoform expression of the mouse, rat, and human Cbfa1/Osf2 transcription factor. Gene 214(1–2):187–197.

Xiao G, Jiang D, Thomas P, Benson MD, Guan K, Karsenty G, et al. 2000. MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. J Biol Chem 275(6):4453–4459.

Xu J, Rodriguez D, Petitclerc E, Kim JJ, Hangai M, Moon YS, et al. 2001. Proteolytic exposure of a cryptic site

within collagen type IV is required for angiogenesis and tumor growth in vivo. J Cell Biol 154(5):1069–1079.

Yang J, Fizazi K, Peleg S, Sikes CR, Raymond AK, Jamal N, et al. 2001. Prostate cancer cells induce osteoblast differentiation through a Cbfa1-dependent pathway. Cancer Res 61(14):5652–5659.

Yee D, Paik S, Lebovic GS, Marcus RR, Favoni RE, Cullen KJ, et al. 1989. Analysis of insulin-like growth factor I gene expression in malignancy: Evidence for a paracrine role in human breast cancer. Mol Endocrinol 3(3):509–517.

Yu Q, Stamenkovic I. 2000. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 14(2):163–176.

Zhang J, Dai J, Qi Y, Lin DL, Smith P, Strayhorn C, et al. 2001. Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. J Clin Invest 107(10):1235–1244.

Zhau HY, Chang SM, Chen BQ, Wang Y, Zhang H, Kao C, et al. 1996. Androgen-repressed phenotype in human prostate cancer. Proc Natl Acad Sci USA 93(26):15152–15157.

Zheng DQ, Woodard AS, Fornaro M, Tallini G, Languino LR. 1999. Prostatic carcinoma cell migration via alpha(v)beta3 integrin is modulated by a focal adhesion kinase pathway. Cancer Res 59(7):1655–1664.

Zidar N, Gale N, Kambic V, Fischinger J. 2002. Proliferation of myofibroblasts in the stroma of epithelial hyperplastic lesions and squamous carcinoma of the larynx. Oncology 62(4):381–385.