Neoplasia • Vol. 4, No. 3, 2002, pp. 191–194

www.nature.com/neo

The Role of $\alpha_v\beta_3$ in Prostate Cancer Progression[1]

Carlton R. Cooper, Christopher H. Chay and Kenneth J. Pienta

Department of Internal Medicine, University of Michigan, Comprehensive Cancer Center, Ann Arbor, MI 48109, USA

Abstract

Integrin $\alpha_v\beta_3$ is involved in varied cell biological activities, including angiogenesis, cell adhesion, and migration on several extracellular matrix components. Although $\alpha_v\beta_3$ is not typically expressed in epithelial cells, it is expressed in macrophages, activated leukocytes, cytokine-stimulated endothelial cells, osteoclasts, and certain invasive tumors. Interestingly, the adhesion and migration of breast cancer cells on bone matrix are mediated, in part, by $\alpha_v\beta_3$. Similar to breast cancer cells, prostate cancer cells preferentially metastasize to the bone. The biological events that mediate this metastatic pattern of prostate cancer are not well defined. This review discusses the role $\alpha_v\beta_3$ plays in prostate cancer progression, with specific emphasis on bone metastasis and on $\alpha_v\beta_3$ signaling in prostate cancer cells. The data suggest that $\alpha_v\beta_3$, in part, facilitates prostate cancer metastasis to bone by mediating prostate cancer cell adhesion to and migration on osteopontin and vitronectin, which are common proteins in the bone microenvironment. These biological events require the activation of focal adhesion kinase and the subsequent activation of PI-3 kinase/Akt signaling pathway.

Neoplasia (2002) **4**, 191–194 DOI: 10.1038/sj/neo/7900224

Keywords: bone metastasis, human prostate cancer cells, $\alpha_v\beta_3$, extracellular matrix, PI-3/Akt pathway.

Introduction

Integrins play a significant role in prostate tumor progression [1]. Schmelz et al. demonstrated that well-differentiated tumors had a higher expression of α_3 and α_6 integrins compared to anaplastic tumors. This study also reported that an increase in $\alpha_6\beta_1$ integrin expression on prostate cancer cells was associated with invasion of the seminal vesicles. The expression of integrin $\alpha_v\beta_3$ was not monitored in this study [1]. Unfortunately, the information regarding the role of $\alpha_v\beta_3$ in prostate tumorigenesis and metastasis is limited. This review will infer from the literature the potential role this integrin plays in prostate tumor progression.

Integrin $\alpha_v\beta_3$ mediates cell adhesion and migration on a variety of extracellular matrix (ECM) proteins, including vitronectin, fibronectin, fibrinogen, laminin, collagen, osteopontin, and others [2]. It is involved in osteoclast adhesion to bone matrix components osteopontin and bone sialoprotein (BSP) and subsequent degradation of the bone [3,4]. This integrin also mediates osteoblast adhesion to osteopontin at the site of bone resorption [5]. The adhesion of breast cancer cells to bone matrix and the migration of breast cancer cells in BSP are mediated by $\alpha_v\beta_3$ [6,7]. Glioblastoma multiforme adhesion to fibronectin and vitronectin is also mediated by $\alpha_v\beta_3$ [8]. The expression of $\alpha_v\beta_3$ has been detected on macrophages, activated leukocytes, cytokine-stimulated endothelial cells, osteoclasts, and certain invasive tumors; however, it is not commonly expressed in epithelial cells [2]. Angiogenesis facilitates the growth and metastasis of solid tumors by, providing nutrients to the expanding mass and providing a pathway for tumor cell dissemination. The expression of $\alpha_v\beta_3$ on endothelial cells plays an important role in this process [2]. Endothelial cells stimulated by tumor-derived angiogenic factors enter the cell cycle and express the integrin $\alpha_v\beta_3$, which allows endothelial cells to interact with a wide variety of ECM proteins as they invade the tissue surrounding the tumor. Antagonists to this integrin induce apoptosis specifically in angiogenic endothelial cells, thereby facilitating regression in several tumors including breast [9-11].

One critical function of $\alpha_v\beta_3$ is to inhibit endothelial cell apoptosis during angiogenesis. The ligation of endothelial $\alpha_v\beta_3$ to osteopontin and vitronectin inhibits apoptosis in endothelial cell cultures upon serum withdrawal. This $\alpha_v\beta_3$-mediated activity requires the activation of NF-κB because nonphosphorylatable IκB completely blocks the survival effect of osteopontin and vitronectin [12]. Nonphosphorylated IκB binds NF-κB in the cytoplasm. When properly stimulated, IκB is phoshorylated, ubiquinated, and degraded, thereby releasing NF-κB to translocate to the nucleus and activate specific genes. In endothelial cells, NF-κB stimulates the expression of osteoprotegerin, which has been implicated as a cell survival factor due to its interaction with TNF-related apoptosis-inducing ligand. The mechanism used by osteoprotegerin to protect endothelial cells from apoptosis induced by serum withdrawal is not known [13].

Address all correspondence to: Dr. Carlton R. Cooper, Department of Internal Medicine, University of Michigan Comprehensive Cancer Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0946, USA. E-mail: cacooper@umich.edu

[1]This work was supported by the Specialized Program of Research Excellence (SPORE) grant in prostate cancer at The University of Michigan Comprehensive Cancer Center ref no. P50 CA 69568 and Comprehensive Cancer Center Grant ref no. CA 46592. Cooper is supported by a 1999 American Foundation for Urological Disease Fellowship (Zeneca Pharmaceuticals). Pienta is supported, in part, by CaPCURE.

Received 13 June 2001; Accepted 25 July 2001.

The Expression of $\alpha_v\beta_3$ on Prostate Cancer Cell Lines

The surface expression of $\alpha_v\beta_3$ is established in commonly used prostate cancer cell lines, including DU145, PC-3, and TSU [14,15]. The expression of $\alpha_v\beta_3$ on LNCaP cells is in dispute. Although Witkowski et al. [15] detected both α_v and β_3 integrin subunits in LNCaP cells, Zheng et al. [16] demonstrated that LNCaP cells did not express heterodimer $\alpha_v\beta_3$. These conflicting results may be due to the types of antibodies used in each study. The former study [15] used antibodies to the monomeric α_v and β_3 subunits, whereas the latter study [16] used antibodies that recognize the $\alpha_v\beta_3$ heterodimer. In addition to the previously mentioned prostate cancer cell lines, Putz et al. [17] established four prostate cancer cell lines that were derived from bone marrow aspirates and demonstrated that all four cell lines expressed α_v and β_3 integrin subunits. Interestingly, breast and lung cancer cell lines that were also derived from bone marrow aspirates also expressed $\alpha_v\beta_3$, suggesting that it may play a role in cancer cell metastasis to the bone marrow.

The Function of $\alpha_v\beta_3$ in Prostate Cancer Cells

The adhesion of cancer cells to the vascular endothelium is a critical step in the metastatic cascade and is mediated by integrins [18,19]. Romanov and Goligorsky [20] used phage display to identify integrins that are important mediators of prostate cancer cell adhesion to interleukin-1–stimulated human umbilical vein endothelial cells (HUVECs). Their data demonstrated that $\alpha_v\beta_3$, along with $\alpha_5\beta_1$ and $\alpha_3\beta_1$, mediated PC-3 and DU145 adhesion to HUVEC monolayers. Another study showed that murine melanoma cell adhesion to microvascular endothelium stimulated by eicosanoid 12(*S*)-hydroxyeicosatetraenoic acid was mediated solely by $\alpha_v\beta_3$ [21]. However, because Romanov and Goligorsky used HUVEC, the relevance of their study to clinical prostate cancer is questioned because prostate cancer frequently metastasizes to the bone. In addition, prostate cancer cell lines have been shown to adhere preferentially to human bone marrow endothelial cells over HUVEC [22]. While $\alpha_v\beta_3$ may be important for prostate cancer cell adhesion to HUVEC, the role this integrin plays in prostate cancer cell adhesion to bone marrow endothelium has not been determined.

Prostate cancer cell adhesion to and migration on components present in the bone matrix are mediated, in part, by $\alpha_v\beta_3$ [23]. A well-characterized antibody to $\alpha_v\beta_3$ (LM609) reduced DU145 cell adhesion to crude bone protein extract by 94%. The protein makeup of the crude bone protein extract was not determined; however, osteopontin and vitronectin are common proteins in mature bone and appropriate ligands for $\alpha_v\beta_3$ [16,24]. Zheng et al. [16] demonstrated that PC-3 cell adhesion and migration to vitronectin and osteopontin were $\alpha_v\beta_3$-dependent. LNCaP cells did not express this integrin and therefore did not adhere to nor migrate on vitronectin and osteopontin. Exogenous expression of $\alpha_v\beta_3$ in LNCaP cells mediated adhesion to vitronectin but not to osteopontin. Surprisingly, the LNCaP cells used in a study performed by Witkowski et al. [15] expressed $\alpha_v\beta_3$, yet these cells did not adhere to vitronectin, suggesting that the monomeric forms of this integrin were not functionally active. LNCaP cell adhesion to osteopontin was not evaluated in that study.

Osteopontin-induced growth of prostate cancer cells in the bone marrow is mediated by $\alpha_v\beta_3$ [25,26]. One study demonstrated that osteopontin stimulated anchorage-independent growth of cell lines LNCaP and C4-2, an androgen-independent subline of LNCaP. Interestingly, osteopontin was detected in greater amounts in androgen-independent prostate cancer cell lines such PC-3 and C4-2, suggesting that it contributes to androgen-independent growth in the bone [25]. Lecrone et al. [26] confirmed this observation by showing that bone-derived osteopontin triggered calcium [Ca^{2+}]-dependent signalling in PC-3 cells. An antibody to $\alpha_v\beta_3$ inhibited these Ca^{2+} signals in PC-3 cells. Fluctuations in intracellular Ca^{2+} regulate signal transduction events that are often associated with cell growth. The fluctuations were not observed in DU145 and LNCaP in the presence of any bone-derived fractions. Interestingly, DU145 cells express α_v and β_3 integrin monomers, but the heterodimer expression of $\alpha_v\beta_3$ is not known. Moreover, secretory products from PC-3 cells but not LNCaP cells upregulated osteopontin expression in MC3T3-E1 cells, an osteoblastic cell line [27]. These observations suggest that prostate cancer cells entering the bone environment are surrounded by osteopontin from a few sources, including the bone matrix, osteoblasts, and the cancer cells themselves. The presence of osteopontin can mediate preferential adhesion, migration, and growth of prostate cancer cells expressing $\alpha_v\beta_3$.

Another investigation demonstrated that $\alpha_v\beta_3$ was involved in bombesin stimulation of prostate cancer cell motility [28]. Neuroendocrine cells in prostate cancer express and secrete bombesin-like peptides, suggesting that these peptides are involved in prostate cancer progression. The study demonstrated that bombesin increased PC-3 cell invasion through matrigel, but did not alter its adhesion to ECM proteins including vitronectin. Bombesin also increased the tyrosine phosphorylation of a 95-kDa protein, which was coimmunoprecipitated with the α_v, β_3, and β_5 integrin subunits. In addition, bombesin treatment caused β_1, β_3, and β_5 integrin subunits to coimmunoprecipitate with focal adhesion kinase (FAK). This suggests that the bombesin signaling is mediated by $\alpha_v\beta_3$ and FAK activation.

The functions of $\alpha_v\beta_3$ in prostate cancer cells are mediated by FAK, which activates the phosphatidylinositol 3-kinase (PI-3 kinase)/Akt pathway [16,24] (Figure 1). Transfection of FAK-related nonkinase, which competes with FAK for its correct localization and phosphorylation, in LNCaP cells previously transfected with $\alpha_v\beta_3$ inhibited the migration of these cells on vitronectin. FAK was also activated in PC-3 cells adherent to vitronectin and $\alpha_v\beta_3$-transfected LNCaP adherent to osteopontin [16,24]. Although FAK phosphorylation was not evaluated in PC-3 cells adherent to osteopontin, one can speculate that FAK was phosphorylated because the PI-3 kinase/Akt pathway was activated in these cells as well [16].

The role of the PI-3 kinase/Akt pathway in prostate cancer cell adhesion and migration on both osteoponitin and

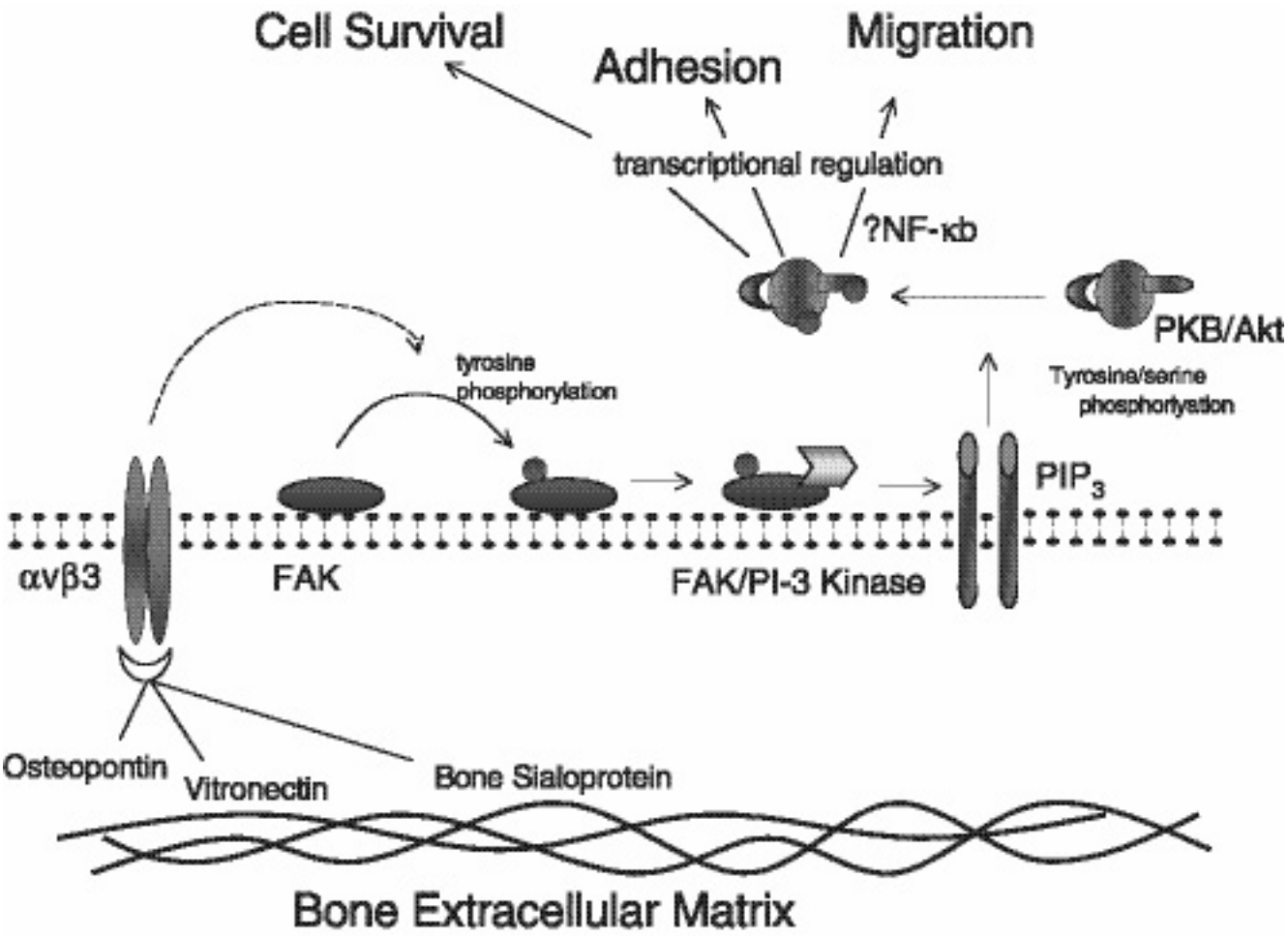

Figure 1. *$\alpha_v\beta_3$ signaling pathway in prostate cancer cells. Ligation of $\alpha_v\beta_3$ with multiple ligands (i.e., osteopontin, BSP, or vitronectin) activates FAK, which interacts and activates PI-3 kinase [23]. The products of PI-3 kinase activity recruit PKB/Akt to the cell membrane where it is activated by phosphorylation and it phosphorylates several substrates to elicit a variety of biological responses, including cell survival, adhesion, and migration. Although NF-κB is potentially involved in the $\alpha_v\beta_3$ signal transduction pathway [8], its role in this signaling pathway is not known for prostate cancer cells.*

vitronectin has been demonstrated by using wortmanin, a specific PI-3 kinase inhibitor. This compound inhibited PC-3 cell adhesion to and migration on both substrates [16]. The PI-3 kinase/Akt pathway may also be involved in androgen-independent growth of prostate cancer cells described earlier [25]. Once activated by an upstream kinase such as FAK, this pathway facilitates cell survival and proliferation by increasing cell cycle regulator E2F, which mediates progression through the cell cycle, and by preventing the proapoptotic activity of BAD. The phosphorylation of BAD by Akt-associated protein kinase B (PKB) prevents it from heterodimerizing with Bcl-2 or Bcl-X_L. These well-known antiapoptotic proteins may be able to provide survival signals that protect prostate cancer cells from apoptosis induced by various stresses like hormone ablation and chemotherapy [29,30]. FAK also activates NF-κB, which is known to regulate the transcription of antiapoptotic proteins [30].

Summary

Cell adhesion molecules (CAMs) are important for the preferential metastasis of prostate cancer to bone [19,22,31]. These CAMs mediate the initial adhesion to the human bone marrow endothelium [22,31] and then the underlying bone matrix [19]. Studies have shown that $\alpha_v\beta_3$ mediates prostate cancer cell adhesion to and migration on vitronectin and osteopontin, which are components of the bone microenvironment [16]. This integrin also mediates osteopontin-associated androgen-independent growth of prostate cancer cells, a common characteristic of advanced prostate cancer [25]. Ligation of $\alpha_v\beta_3$ with its respective ligands activates FAK and subsequently the PI-3 kinase/Akt pathway, resulting in a variety of biological effects, including suppression of apoptosis [29]. Although it is not known if direct engagement of $\alpha_v\beta_3$ in prostate cells prevents apoptosis through the PI-3 kinase/Akt pathway [16], the data presented suggest that $\alpha_v\beta_3$ through this signal pathway may be involved in prostate cancer metastasis to bone.

Acknowledgement

We gratefully acknowledge Kathleen Woods Ignatoski for her insight in writing this review.

References

[1] Schmelz M, Cress AE, Scott KM, Burger F, Cui H, Sallam K, McDaniel KM, Dalkin BL, and Nagle RB (2002). Different phenotypes in human prostate cancer: α6 or α 3 integrin in cell-extracellular adhesion sites. *Neoplasia* **4**, 243–54.

[2] Varner JA, and Cheresh DA (1996). Tumor Angiogenesis and the Role of Vascular Cell Integrin $\alpha_v\beta_3$, in Important advances In Oncology VT DeVita, S Hellman, and SA Rosenberg (Eds) Lippincott-Raven Publishers, Philadelphia, PA. pp. 69–87.

[3] Teitelbaum SL, and Ross FP (1994). Mechanisms of tumor-induced osteolysis. *Lab Invest* **71**, 453–55.

[4] Ross FP, et al. (1993). Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alphaV-beta3 potentiate bone resorption. *J Biol Chem* **268**, 9901–907.

[5] Cheng S-L, et al. (2000). Regulation of alphaVbeta3 and alphaVbeta5 integrins by dexamethasone in normal human osteoblastic cells. *J Cell Biochem* **77**, 265–76.

[6] vanderPluijm G, Vloedgraven H, Papapoulos S, Lowik C, Grzesik W, Kerr J, and Robey PG (1997). Attachment characteristics and involvement of integrins in adhesion of breast cancer cell lines to extracellular bone matrix components. *Lab Invest* **77**, 665–75.

[7] Sung V III, JTS, Fisher L, Aaron AD, and Thompson EW (1998). Bone sialoprotein supports breast cancer cell adhesion proliferation and migration through differential usage of the alpha(v)beta3 and alpha(v)beta5 integrins. *J Cell Physiol* **176**, 482–94.

[8] Richie CK, Giordano A, and Khalili K (2000). Integrin involvement in glioblastoma multiforme: possible regulation by NF-kappaB. *J Cell Physiol* **184**, 214–21.

[9] Brooks PC, Montgomery AMP, Rosenfeld M, Reisfeld RA, Hu T, Klier G, and Cheresh DA (1994). Integrin $\alpha_v\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* **79**, 1157–64.

[10] Brooks PC, Clark RAF, and Cheresh DA (1994). Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. *Science* **264**, 569–71.

[11] Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, and Cheresh DA (1995). Antiintegrin $\alpha_v\beta_3$ blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest* **96**, 1696–97.

[12] Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, and Giacheli CM (1998). NF-κB mediates alphaV-beta3 integrin–induced endothelial cell survival. *J Cell Biol* **141**, 1083–93.

[13] Malyankar UM, Scatena M, Suchland KL, Yun TJ, Clark EA, and Giachelli CM (2000). Osteoprotegerin is an alphaV beta 3–induced, NF-κB–dependent survival factor for endothelial cells. *J Biol Chem* **275**, 20959–62.

[14] Haywood-Reid PL, Zipf DR, and Springer WR (1997). Quantification of integrin subunits on human prostatic cell lines — comparison of nontumorigenic and tumorigenic lines. *Prostate* **31**, 1–8.

[15] Witkowski CM, Rabinovitz I, Nagle RB, Affinito K-SD, and Cress AE (1993). Characterization of integrin subunits, cellular adhesion and tumorgenicity of four human prostate cell lines. *J Cancer Res Clin Oncol* **119**, 637–44.

[16] Zheng D-Q, Woodard AS, Tallini G, and Languino LR (2000). Substrate specificity of $\alpha_v\beta_3$ integrin–mediated cell migration and phosphatidylinositol 3-kinase/AKT pathway activation. *J Biol Chem* **275**, 24565–74.

[17] Putz E, Witter K, Offner S, Stosiec P, Zippelius A, Johnson J, Zahn R, and Pantel GRK (1999). Phenotypic characteristics of cell lines derived form disseminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases. *Cancer Res* **59**, 241–48.

[18] Honn KV, and Tang DG (1992). Adhesion molecules and tumor cell interaction with endothelium and subendothelial matrix. *Cancer Metastasis Rev* **11**, 353–75.

[19] Cooper CR, and Pienta KJ (2000). Cell adhesion and chemotaxis in prostate cancer metastasis to bone: a minireview. *Prostate Cancer Prostatic Dis* **3**, 6–12.

[20] Romanov VI, and Goligorsky MS (1999). RGD-recognizing integrins mediate interactions of human prostate carcinoma cells with endothelial cells *in vitro*. *Prostate* **39**, 108–18.

[21] Tang DG, Diglio CA, and Honn KV (1994). Activation of microvascular endothelium by eicosanoid 12(*S*)-hydroxyeicosatetraenoic acid leads to enhanced tumor cell adhesion *via* up-regulation of surface expression of $\alpha_v\beta_3$ integrin: a posttranscriptional, protein kinase C– and cytoskeleton-dependent process. *Cancer Res* **54**, 1119–29.

[22] Lehr JE, and Pienta KJ (1998). Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. *J Natl Cancer Inst* **90**, 118–23.

[23] Hullinger TG, McCauley LK, Dejoode ML, and Somerman MJ (1998). Effect of bone proteins on human prostate cancer cell lines *in vitro*. *Prostate* **36**, 14–22.

[24] Zheng D-Q, Woodard AS, Fornaro M, Tallini G, and Languino LR (1999). Prostatic carcinoma cell migration via $\alpha_v\beta_3$ integrin is modulated by a focal adhesion kinase pathway. *Cancer Res* **59**, 1655–64.

[25] Thalmann G, Sikes R, Devoll R, Kiefer J, Markwalder R, Klima I, Farach-Carson C, Studer U, and Chung L (1999). Osteopontin: possible role in prostate cancer progression. *Clin Cancer Res* **5**, 2271–77.

[26] Lecrone V, Li W, Devoli RE, Logothetis C, and Farach-Carson MC (2000). Calcium signals in prostate cancer cells: specific activation by bone-matrix proteins. *Cell Calcium* **27**, 35–42.

[27] Hullinger TG, Taichman RS, Linseman DA, and Somerman MJ (2000). Secretory products from PC-3 and MCF-7 tumor cell lines upregulate osteopontin in MC3T3-E1 cells. *J Cell Biochem* **78**, 607–16.

[28] Aprikian AG, Tremblay L, Han K, and Chevalier S (1997). Bombesin stimulates the motility of human prostate-carcinoma cells through tyrosine phosphorylation of focal adhesion kinase and of integrin-associated proteins. *Int J Cancer* **72**, 498–504.

[29] Downward J (1998). Mechanisms and consequences of activation of protein kinase B/Akt. *Curr Opin Cell Biol* **10**, 262–67.

[30] Sonoda Y, Watanbe S, Matsumoto Y, Aizu-Yokota E, and Kasahara T (1999). FAK is the upstream signal protein of the phosphatidylinositol 3-kinase–Akt survival pathway in hydrogen peroxide–induced apoptosis of a human glioblastoma cell line. *J Biol Chem* **274**, 10566–70.

[31] Cooper CR, McLean L, Walsh M, Taylor J, Hayasaka S, Bhatia J, and Pienta KJ (2000). Preferential adhesion to prostate cancer cells to bone is mediated by binding to bone marrow endothelial cells as compared to extracellular matrix components *in vitro*. *Clin Cancer Res* **6**, 4839–47.