

## Review

# Molecular mechanisms of metastasis in prostate cancer

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### Abstract

Prostate cancer (PCa) preferentially metastasizes to the bone marrow stroma of the axial skeleton. This activity is the principal cause of PCa morbidity and mortality. The exact mechanism of PCa metastasis is currently unknown, although considerable progress has been made in determining the key players in this process. In this review, we present the current understanding of the molecular processes driving PCa metastasis to the bone.

*Asian Journal of Andrology* advance online publication, 1 December 2008; doi: 10.1038/aja.2008.29

**Keywords:** bone, bone marrow stroma, metastasis, prostate cancer

### 1 Metastatic mechanisms in the primary tumour

The principal problem arising from prostate cancer (PCa) is its propensity to metastasize. This tendency arises from specific molecular mechanisms and interactions that together lead to local invasion, extravasation and distal migration from the primary site, followed by endothelial attachment, transmigration and site-specific establishment of metastases at secondary sites. Basic knowledge related to this structured process has improved recently, but many of the key elements are still poorly understood.

Local invasion is one of the fundamental early steps in metastasis, as without it tumour spread cannot occur. To develop invasive potential, the malignant cell must down-regulate its cell–cell and cell–matrix adhesive characteristics, become motile and acquire the ability to break down the extracellular matrix (ECM) using degradative enzymes [1]. Once the malignant cell has reached the interstitium, it must enter the vascular or lymphatic circulation by breaching the endothelial barriers. From there, the cell must migrate via the blood or lymphatic circulation and

arrest at a secondary endothelial site before binding to the endothelium, extravasating and transmigrating through the endothelial layer to reach the interstitium, where it proliferates and/or coalesces with other metastasized cells to form a micro-metastasis (Figure 1) [2]. It will do this only if the environment at the secondary site is favourable.

### 2 Primary site cell–cell adhesion

Maintenance of organic architecture depends on cell–cell and cell–matrix binding. In the prostate and other structures, a key cell–cell binding regulator is the cadherin–catenin complex, whereas cell–matrix binding is largely mediated by integrins, dimeric binding proteins comprising  $\alpha$ - and  $\beta$ -chain subunits.

Cadherins are transmembrane glycoproteins, of which E-cadherin is the best characterized in PCa. It serves critical functions during embryogenesis and organogenesis through intercellular adhesion and signaling [3]. The locus coding for E-cadherin (16q22.1) is considered to be a tumour-suppressor gene; loss of function enables cell detachment and induces an invasive phenotype [4], whereas transfection of E-cadherin complementary DNA (cDNA) into invasive adenocarcinoma cells renders them non-invasive [5, 6].

E-cadherin is attached intracellularly to the actin cytoskeleton via intracellular catenin. Once anchored, the transmembrane cadherins bind through their external

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Received: 10 October 2008 Accepted: 11 October 2008

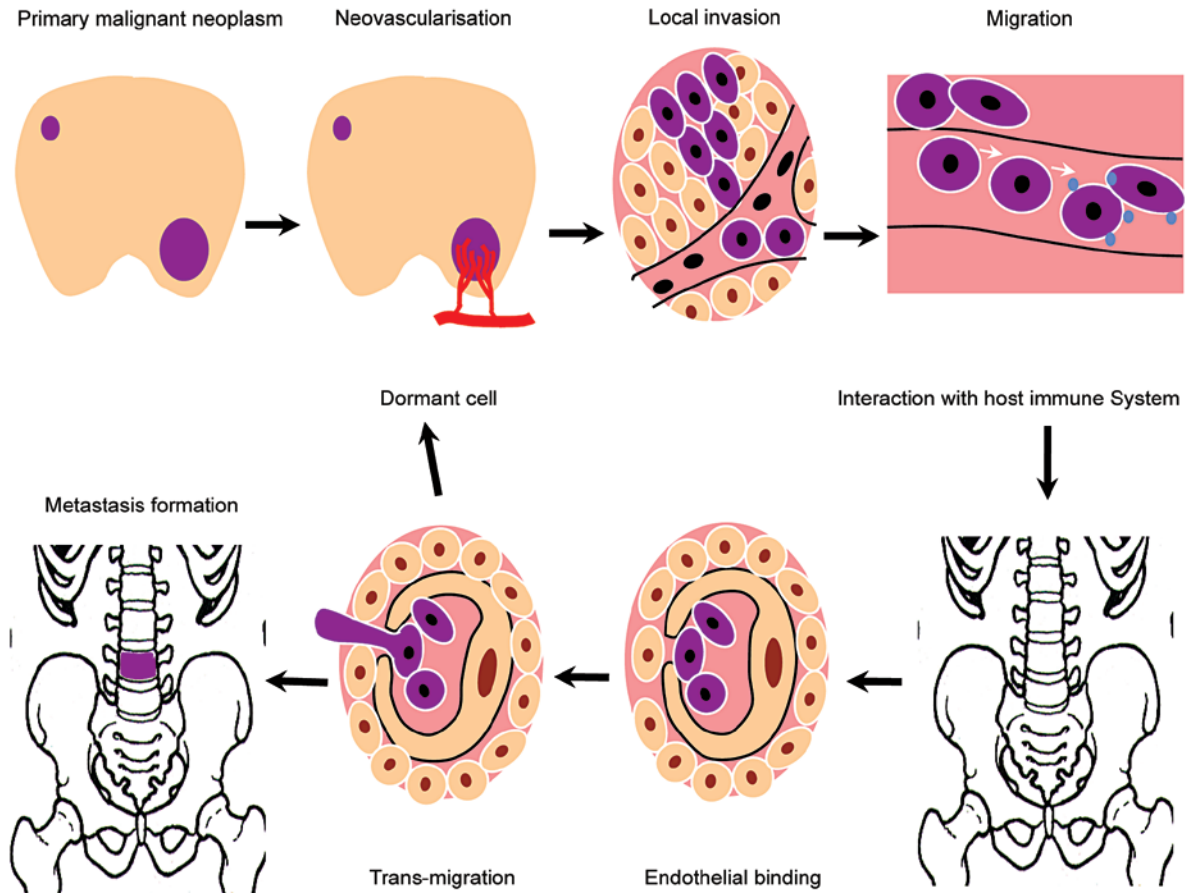


Figure 1. Metastasis is characterized by proliferation, neovascularization and extravasation at the primary site. In the circulation, malignant cells interact with the host immune system, typically resulting in cancer cell destruction or apoptosis. Surviving cells arrest at secondary endothelial sites by a process of lectin binding consolidated by integrin-based stabilization of the epithelial–endothelial binding. The cell then undergoes active transmigration. The binding process is complete within 30–60 min and transmigration within 24 h. Once the cell reaches the interstitium, it may remain dormant for an undefined period or it may coalesce with other cells and proliferate to form a metastatic colony. This will then disturb local physiological function, leading to physiological dysfunction and anatomical disruption. Any metastatic site may produce further metastases (Reproduced with permission from [2]).

domains to the binding sites of other cadherins on adjacent cells. The cadherin–catenin complex is essential for both morphogenesis [7] and subsequent structural and functional organization of epithelia [8], and the disruption of either of the interactive components produces significant alterations in cellular behaviour. E-cadherin has been extensively studied in human cancers, resulting in its nomination as a marker for metastatic biopotential in many tumours [9]. In primary PCa, reduced E-cadherin expression has been correlated with increased tumour grade or stage, and with bone metastasis and poor prognosis [10–12]. Further data have confirmed the correlation with tumour grade, but one study found no relationship between E-cadherin and tumour progression or PCa death [13]. In animal models, low E-cadherin expression has also been described in

both metastasizing and non-metastasizing PCa tumour sublines [5]. An archival study of this issue [14] in paired primary prostate tissue and prostatic bone metastases from the same patients showed decreased expression of E-cadherin messenger RNA (mRNA) in metastases in nine of the total number of cases. The results suggest that E-cadherin down-regulation, although important, is not the foremost step in the metastatic cascade, but this protein is a clinically relevant invasion–metastasis suppressor. Indeed, it is a critical component in the general process of epithelial to mesenchymal transition (EMT). For epithelial cancer to progress and metastasize, cells must undergo this transition, whereby cell polarity and cell–cell binding are lost. These cells assume a mesenchymal phenotype, which gives them the ability to invade the ECM and migrate to distant

sites. This process involves the disruption of stable E-cadherin binding, a primary event governing EMT, and it is accompanied by increased expression of mesenchymal N-cadherin. The EMT process may be an ‘on–off’ phenomenon, and it is possible that transient functional down-regulation of E-cadherin may be a feature of the metastatic process in PCa, with differences in expression at the primary and secondary sites [14].

Integrity of the cadherin–catenin complex and its anchorage to the actin cytoskeleton are required for E-cadherin-mediated intercellular adhesion. Absence or dysfunction of the catenin component of this complex may lead to impaired cellular adhesion, despite apparently normal E-cadherin levels [14, 15]. Clinical studies of PCa confirm the correlation between catenin subtype expression with tumour dedifferentiation and local stage [16], although aberrant expression of  $\alpha$ -catenin is rare in the presence of normal E-cadherin expression. A study of 28 prostatic tumours found consistent abnormalities of E-cadherin and down-regulation of  $\alpha$ -catenin [17], and although Umbas *et al.* [18] detected these effects in only four out of 52 cases, the combination occurred in patients with advanced disease.

$\beta$ -Catenin has dual functions in prostatic and other tissues. In addition to its role in the cadherin–catenin complex, it also regulates signal transduction by binding to DNA and activating gene transcription. Few reports describe abnormal  $\beta$ -catenin signalling as a master regulator of PCa (< 4% of primary prostate tumours have  $\beta$ -catenin mutations [19]), but aberrant  $\beta$ -catenin expression seems to affect the function of the cadherin–catenin complex. This notion is supported in a paired primary or bone metastasis study [14], in which 13 out of 14 primary tumours had high  $\beta$ -catenin expression, whereas 12 out of 14 metastases showed down-regulated  $\beta$ -catenin mRNA levels compared with their primary tumours. Therefore, there is a striking contrast in the levels of  $\beta$ -catenin mRNA between primary tumours and metastases, suggesting a major dysfunction of the cadherin–catenin complex. This factor may be an important early step in the metastatic process. There are, however, unexplained observations that run counter to this hypothesis, for example,  $\beta$ -catenin expression in the primary tumour does not appear to reflect the metastatic potential of tumours in some patients, and E-cadherin is not lost from metastatic cells, although it may be re-expressed in the secondary site once it is lost in the primary site. Better understanding of this process is required, but, overall, these observations suggest that the essential E-cadherin– $\beta$ -catenin complex is often impaired during metastasis.

### 3 Cell–matrix adhesion and matrix degradation

Integrins are essential for cell–matrix attachment. Integrin expression varies between tumours, but over-

expression of  $\alpha_6$  and  $\beta_3$  integrins have been associated with increased invasion [20, 21]. This may suggest the anchorage of the malignant cell to the basement membrane (BM) or the involvement of signalling pathways related to cell motility. Whatever the actual mechanism, integrins are fundamentally important in the binding and migration processes at metastatic sites, where they work together with enzymes that degrade the ECM and BM. These structures are composed mainly of type IV collagen, laminin, fibronectin, entactin and tenascin [22]. Leucocytes and malignant cells are thought to be the only cells that are able to breach the BM, a process facilitated by the production of matrix metalloproteinases (MMPs). Twenty-four MMPs have been described to date [23] and they act to degrade the ECM. MMP activity is regulated by tissue inhibitors of metalloproteinases (TIMPs), and imbalances in the MMP:TIMP ratio due to either TIMP down-regulation or increased MMP production by tumour cells can induce an invasive phenotype [24]. In metastasis, this balance is vitally important both in endothelial barrier degradation [25] and in the establishment of metastases within bone marrow stroma (BMS) [26, 27] (Figure 2). The proteolytic enzyme, urokinase-type plasminogen activator, is also important in the MMP cascade. It has direct lytic activity on fibronectin, and through plasmin it activates procollagenases. It works to initiate MMP action and is particularly critical in the development of PCa metastases [27].

### 4 Cell migration and motility: the GTPase axis

Cell motility and migration in prostate and other cancers are linked integrally to Ras and other GTP-binding proteins, for example, Rho and Rac. These proteins are important for general cellular functions, including cytoskeletal assembly, intracellular signalling and physical movement of cell membranes and whole cells [28]. Ras is a transmembrane glycosylated protein that regulates downstream cellular activities such as cell proliferation, nuclear transcription, apoptosis and invasion [29] (Figure 3). It acts as a membrane transducer, as extracellular signals bind to receptor tyrosine kinases, which in turn activate Ras and initiate downstream events [30, 31]. The Ras family, which has a major influence on cell signalling, comprises *h-ras*, *k-ras*, *n-ras*, *r-ras* and *m-ras*, and although Ras mutations are rare in PCa (3%), they are associated with 30% of solid tumours [31].

The Rho GTPases are similar to Ras in their structure and synthesis; their activation lies downstream of Ras and is therefore Ras-dependent. This family currently comprises RhoA, B, C, E and G; Rac1, Rac2, cdc42-H5 and TC10, all proteins involved in cell motility. It has been suggested [32] that Rho GTPases act through actin dynamics, guiding morphological changes, including cell growth and movement. Cell movement may occur

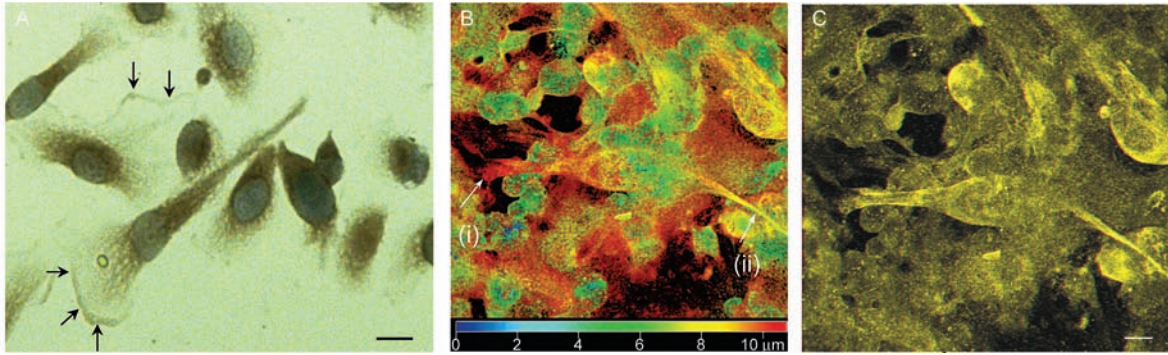


Figure 2. (A): Photomicrograph showing matrix metalloproteinase (MMP)-7 staining of prostate cancer (PCa) cells in culture. High MMP-7 staining is seen at the leading edge of the cell relative to the ruffling border at the margin of the pseudopodial extension (arrows); original magnification ( $\times 400$ ), scale bar = 10  $\mu\text{m}$ . (B): Confocal 3D imaging of PCa cells in bone marrow co-culture showing PCa invasion of bone marrow stroma (BMS). False colour image of a PC-3 cell within the BMS: Blue being 0  $\mu\text{m}$ , closest to the viewer (top of BMS) through to red 10  $\mu\text{m}$ , furthest away from the viewer (bottom of the BMS layer). Using morphology for identification, the arrow (i) shows the leading pseudopodia of a PC-3 cell, with a second arrow (ii) showing the trailing end. (C): 3D image of the same picture showing the PC-3 cell underneath the BMS. MMP concentrations are highest at the leading edge of the cell. The cells also have the extended mesenchymal morphology typical of motile cells. Scale bar = 10  $\mu\text{m}$  (Reproduced with permission from [2]).

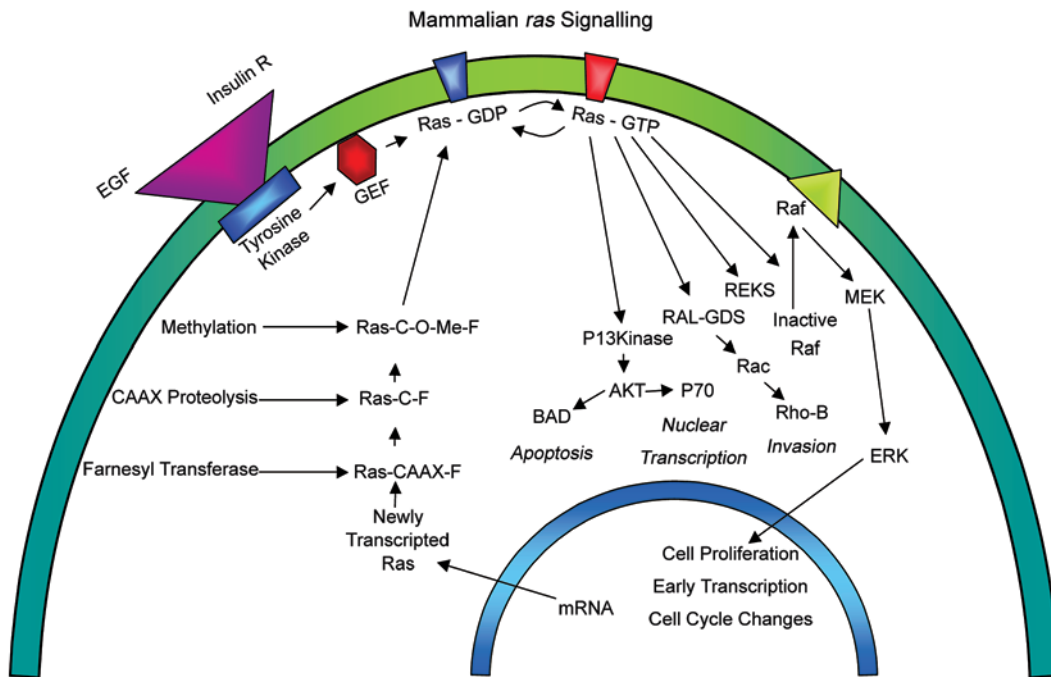


Figure 3. Schematic diagram showing the Ras signalling pathway and its linkage to the GTPase cellular motility axis (Reproduced with permission from [2]).

via a ‘molecular clutch’, which involves the extension of filopodia bound to the cortical actin network and a fixed extracellular ligand, resulting in net movement of the whole cell (Figure 4). Prevention of Rho synthesis or activity should result in reduced cell motility, with a corresponding reduction in invasion across endothelial

barriers. This Ras–Rho-mediated activity is thought to be important in cellular migration and metastasis in prostate and other cancers. A study using bisphosphonates to inhibit the mevalonate pathway (and thus RhoA) in PCa [25] showed that cell motility and transmigration of PCa cells across human bone marrow endothelial (BME)

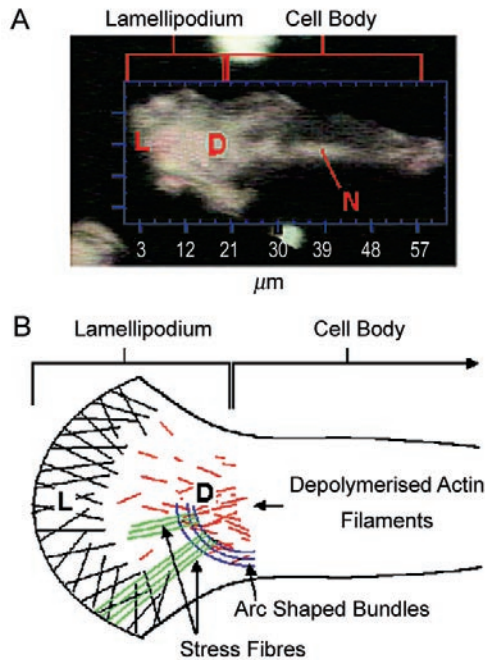


Figure 4. (A): Optical image of a motile prostate cancer (PCa) cell with a lamellipodial extension (L) projecting out in the direction of cellular travel. N, Nucleus; D, de-polymerized actin filaments. (B): Schematic showing the internal architecture of the lamellipodium (L), with actin filaments linking the internal cellular structure to the external lipid cellular membrane. These filaments undergo a constant process of polymerization and destruction (D), resulting in movement of the lamellipodium and forward movement of the cell (Reproduced with permission from [2]).

barriers and human BMS were inhibited in the presence of zoledronic acid. A further study that examined the effects of inhibiting the farnesyl and geranyl-geranyl prenylation pathways [33] showed that migration and motility of PCa cells were reduced dramatically by inhibition of Ras prenylation (and therefore also inhibition of Rho activation). It is likely that the Ras-Rho axis is activated in PCa metastasis and that this underpins the acquisition of cell motility that is fundamental for successful metastasis.

## 5 Prostate tumour cell clearance from peripheral blood

In solid tumours, malignant cells enter the circulation increasingly as the tumour load grows. Iatrogenic cellular shedding into the circulation occurs in clinical situations, for example, during transurethral resection of the prostate (TURP) [34, 35], radical prostatectomy [36], prostate biopsy [37] and brachytherapy [38]. This cellular dissemination is unexpectedly not associated with a perceptible increase in metastasis development, perhaps because of the inability of individual cells to propagate or

because of other unknown factors. Whatever the reality, tumour growth is accompanied by an ongoing process of cellular clearance from the circulation. Some authors suggest that this cell clearance takes up to 4 weeks [37], but this proposed time scale is far too long and the speed of cell clearance from the circulation is almost certainly more rapid. Chambers *et al.* [26] proposed that clearance is mainly attributable to the arrest of relatively large epithelial cells (or cellular clumps) in the first capillary bed they encounter. However, this cannot be the sole explanation; if it were, the incidence of pulmonary and hepatic metastases would be much higher than it actually is in PCa and other cancers. There must be additional relevant factors, predicated on the differential binding of PCa cells and differences in chemo-attraction, that activate cellular motility. *In vitro* models of prostate epithelial cell (PEC) binding to the human BME have shown that the process of epithelial-endothelial binding is virtually complete within an hour. Once this has occurred, epithelial cell migration through the endothelial barrier occurs within 24 h [39] (Figure 5). These laboratory findings are supported by reverse transcriptase-polymerase chain reaction-based measurements in men undergoing TURP, showing that PECs appear in the circulation upon commencement of surgery, but are undetectable within 2 h of the procedure's conclusion [40]. It is clear, therefore, that once a cell enters the circulation, it is rapidly taken out, probably by endothelial surface binding at a secondary site.

## 6 Distal attachment and tumour cell transmigration through the endothelium

Tumour cells arrest on endothelial surfaces within the circulatory system and subsequently undergo transendothelial

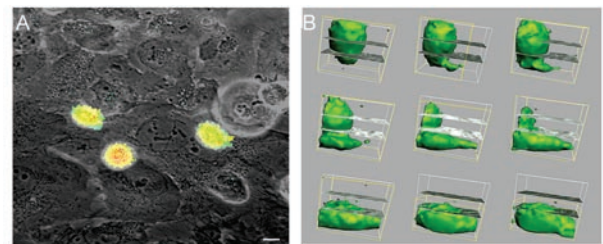


Figure 5. (A): Photomicrograph of a bone marrow endothelial (BME) monolayer (phase contrast) seeded with prostate cancer (PCa) PC-3 cells transfected with green fluorescent protein (GFP) (green). Cells bind to the junctional endothelial areas within 30–60 min. Thereafter they induce BME retraction and migrate into the interstitium. Scale bar = 10 μm. (B): This process involves active cellular movement and cellular expansion as shown by the time-lapse volumetric reconstructions of GFP-marked cells as they extravasate through the endothelial monolayer (Reproduced with permission from [2]).

migration; this is a key event in cancer metastasis. Tumour cell–endothelial interactions involve multiple adhesive interactions (docking and locking) at the molecular level [21]. The initial step is thought to involve selectins, followed by stabilization through integrin binding [41]. These are not the only binding steps, because antibodies to CD11a, CD18, LFA-1 and CD31 have been shown to interfere with the binding process [42].

Site-specific adhesion determinants play a role in preferential metastasis to individual organs. Molecules postulated to be involved in tumour–endothelial adhesion include platelet–endothelial cell adhesion molecule-1 (PECAM-1 or CD31) [43],  $\alpha_4\beta_1$  integrin and sialyl Lewis X, which bind to the endothelial cells through E-selectin, vascular cell adhesion molecule 1 (VCAM-1) [44] and others.

Tumour cells penetrate endothelial junctions after adhering to the surface of endothelial cells (Figures 5 and 6). Endothelial cells appear to be actively involved in transmigration, as dynamic changes occur in the expression and localization of adhesion molecules, including N-cadherin, VE-cadherin and PECAM-1 [45], inducing endothelial cell retraction once the tumour cell adheres to the underlying ECM. Binding to laminin, type IV and type V collagens is mediated by  $\beta_1$  and  $\beta_4$  integrins, whereas binding to hyaluronan, fibronectin, type I collagen and cellular migration is mediated by  $\beta_1$  integrins and CD44 [44, 46–49]. Understanding the process of secondary site binding and endothelial transmigration in PCa has been facilitated by the development of co-culture models using human BMS and primary PECs and PEC lines [27, 39] and the establishment of prostate epithelial colonies in human BMS [50, 51]. These models have demonstrated that cell–matrix binding depends fundamentally on the  $\beta_1$  integrin component of the integrin-binding mechanism. Studies using various endothelial types as well as benign

and malignant PECs have shown that PECs bind more avidly to bone marrow endothelial cells (BMECs) than to other endothelia and that benign and malignant cells have the same binding capacity for those endothelial surfaces. Why, then, do metastases not develop from the PECs known to be present in the circulation during prostatic resection for benign prostatic hyperplasia (BPH)? The answer to this question lies in the differential ability of PECs to migrate across the endothelial barrier. *In vitro* studies using green fluorescent protein (GFP)-transfected PCa cells in conjunction with time-lapse confocal microscopy have enabled cellular tracking measurements of benign and malignant PECs in epithelial–endothelial co-culture [39] (Figures 5 and 6) and have shown that only malignant cells will transmigrate through the endothelial layer. Benign cells will bind in the same way as malignant cells, but they do not cross the endothelial barrier into the interstitium [39].

Explication of this mechanism is critical to the understanding and potential treatment of metastases in PCa. Once bound, PECs induce rapid endothelial cell retraction (Figure 6), but the precise mechanism inducing this reaction is at present unclear. A major component of the signalling cascade modulating endothelial permeability is intracellular  $\text{Ca}^{2+}$  [52]. Studies by Lewalle *et al.* [53] showed that binding of breast epithelial cells to human umbilical vein endothelium cells (HUVECs) induced a transitory rise in the HUVEC intracellular  $\text{Ca}^{2+}$  concentration, resulting in endothelial retraction and epithelial migration. This  $\text{Ca}^{2+}$  rise and the process of endothelial retraction are entirely dependent on cell–cell contact, and inhibition of the  $\text{Ca}^{2+}$  elevation inhibited breast epithelial cell transendothelial migration. Binding of PECs and melanoma cells also induces increases in intracellular  $\text{Ca}^{2+}$  levels [54], correlating with increased binding of the epithelial cells. Further studies of calcium-binding agents support this notion:

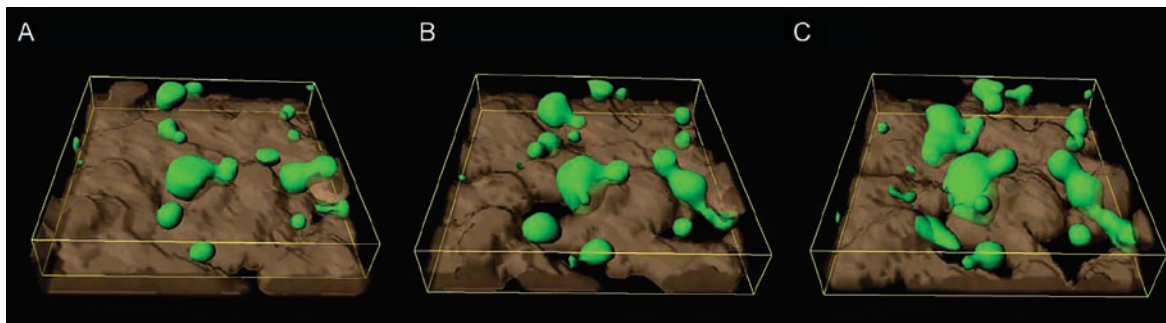


Figure 6. Reconstructed confocal microscopic image of prostate cancer (PCa) cells transfected with green fluorescent protein (GFP) (green) seeded onto a bone marrow endothelial (BME) monolayer (grey) and photographed sequentially with time-lapse confocal microscopy. (A): PCa cells binding to the junctional areas of the endothelium. (B): The endothelial cells have started to retract, leaving gaps in the endothelial barrier. (C): The epithelial cells then migrate through into the underlying interstitium (Reproduced with permission from [2])

treatment of BMEC lines with zoledronic acid, a potent calcium-chelating agent, tightens endothelial–endothelial cell binding and limits transmigration [25].

The effect of Ras–Rho inhibition of reducing the propensity of PECs to invade across endothelial barriers suggests that a major component affecting cancer cell migration is inhibition of transduction pathways related to the Rho axis. This inhibitory effect has been demonstrated *in vitro* in PCa using zoledronic acid [25] and the prenylation inhibitor AZD3409 [33, 55]. The inhibitory effects of these compounds are known to be related to Rho through its interaction with Ras [56]. Inhibition of this pathway affects downstream prenylation of small GTPases (Rho–Rac), which are known to have an integral involvement in cell motility. Therefore, an early event following integrin  $\beta_1$  binding in PCa cells may be the induction of specific pathways that relate to Ras and subsequently Rho–Rac. These in turn are associated with the epithelial-to-mesenchymal transition, a phenomenon known to be important in the cellular migration process.

### 7 Chemo-attraction at the secondary site: chemokines and lipids

The ‘seed–soil’ hypothesis of Paget is exemplified by PCa, with its predilection for the red bone marrow of the axial skeleton [57]. Once there, the malignant cells disturb integrated and balanced skeletal functions and displace the red bone marrow, inducing marrow dysfunction and bone marrow failure. Two factors contribute to this homing phenomenon: the presence of chemokines and the affinity for energy-rich sources, such as specific lipids that are freely available within red marrow adipocytes.

The chemokine axis is important in the homing of haematological and immunological cells to specific targets. Cells from various epithelial tumours share many of the trafficking characteristics of this haematopoietic stem cell (HSC) homing system [58]. Homing of the HSCs to the bone marrow during foetal life and after bone marrow transplantation has been well characterized. The key molecular axis for this process was identified as the CXC chemokine stromal-derived factor-1 (SDF-1 or CXCL12) and its receptor CXCR4 (CD186). This model is supported by the knowledge that both BMECs and osteoblasts express SDF-1 [59–61], the observation that CXCR4 knock-outs do not show haematopoietic engraftment of the bone marrow [62] and the recognition that the level of CXCR4 expression in HSCs determines their ability to engraft the bone marrow [63]. The CXCR4–SDF-1 axis is also known to play an important role in targeting solid tumour metastases to the bone marrow. This is important in various primary tumours, including the breast [58], kidney [64], lung [65], pancreas [66] and prostate [57, 67].

*In vitro*, CXCR4 and SDF-1 are involved in the motility process: interactions alongside CCR7 or CCL21 trigger pseudopodial invasion by malignant breast epithelial cells through actin polymerization [58]. These results have led to the hypothesis that CXCR4 is the key component of metastatic implantation in the bone marrow and that it represents an important therapeutic target for metastatic bone disease in PCa and other cancers. Indeed, blockage of CXCR4 signalling in breast cancer by neutralizing antibodies [58] or peptide antagonists such as T140 [68] has been shown to inhibit metastasis *in vivo*. Sun *et al.* [67] also showed that CXCR4 expression increased with increasing prostatic malignancy; the greatest expression was observed in aggressively metastatic PC-3 cells and in human bone metastasis specimens. This gradient of expression suggests that CXCR4–SDF-1 signalling may be a key signalling pathway for metastatic spread to the bone. It has also been demonstrated [57], using a matrigel BM invasion assay, that SDF-1 signalling induced both DU145 and PC-3 cells to invade. However, Hart *et al.* [39] used recombinant SDF-1 and T140 inhibitors to show that the CXCR4–SDF-1 signalling pathway is not the sole chemo-attractant important for the spread of PECs to the bone, confirming that, although SDF-1 is a potent stimulus for invasion, the level of invasion it induces is significantly less than that seen using either BMECs or BMS alone. This phenomenon was reinforced in these experiments by the observation that the use of a specific CXCR4 antagonist peptide (T140), at a concentration that blocked PEC invasion in response to maximum levels of SDF-1 signalling, did not completely block invasion towards either BME or BMS. Thus, although the CXCR4/SDF-1 signalling pathway is important in PCa metastasis, it is not the only chemokine signalling pathway involved [39].

Another important stimulus is the requirement for the metastasizing PCa cells to seek a lipid source. Cancer cells are in a state of rapid metabolism and have a fundamental requirement for lipids, to be used either as an energy source or in the processes involved in tumour cell maintenance, proliferation and migration. An *in vitro* study [69] showed that PC-3 cells grew rapidly in the vicinity of lipid cells in bone marrow, and further studies [70] showed that PCa cells take up lipids rapidly as soon as they are seeded onto the human bone marrow (Figure 7). Specific lipids also act as strong chemo-attractants for PCa cells. Treatment with arachidonic acid, an omega-6 lipid, results in rapid migration of PC-3 cells towards bone marrow stroma, an effect that is blocked competitively using omega-3 lipids. Further experiments of lipid depletion in the bone marrow confirm this effect: the attractiveness of the human bone marrow to PCa cells decreased dramatically once the BMS was depleted of lipid cells prior to epithelial seeding [70], confirming that specific lipids are critical to metastasis

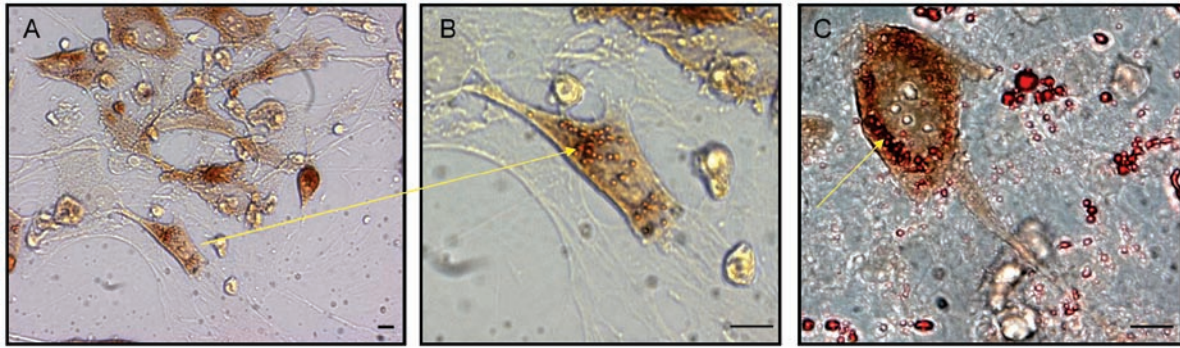


Figure 7. (A): Photomicrograph showing lipid uptake by a prostate cancer (PCa) cell in co-culture. (B): The lipid droplets (Oil Red O-stained vesicles) are taken up rapidly by the PCa cell and have been shown to be intracellular using confocal microscopy. (C): At higher magnification, the lipid droplets are more clearly demonstrated to be inside the PCa cells after co-culture with human BMS. The PCa cells have an affinity for the adipocytes and co-localize with them in the bone marrow. Scale bars = 10  $\mu\text{m}$  (Reproduced with permission from [2]).

and that they may be an important determinant of the site specificity of the bone marrow in PCa.

## 8 Molecular mechanisms of metastatic prostate cancer in the bone or bone marrow

Once established at the secondary site, prostatic micro-metastases develop in the bone marrow space, often in close association with the bone surface, where the osteoblast–fibroblast microenvironment is disturbed locally. It is postulated that the first event in this metastatic developmental process is osteoclast-mediated bone resorption, leading to the release of stimulatory cytokines from the bone surface and inducing a cycle of resorption or tumour stimulation, but this hypothesis has not been proven definitively. As the metastasis develops, an imbalance occurs in the regulated, coupled skeletal cycle of bone resorption and bone formation, resulting in accelerated and synchronous bone formation and resorption. This is caused by changes in local cytokine production and interactions (Figure 8).

Many stimulating factors have been identified with respect to osteoblastic metastases in PCa. These mechanisms have been clarified in recent years and point towards the importance of the endothelin axis. There are three types of endothelin (ET-1, -2 and -3), which act through the endothelin receptors ETa and ETb. They are synthesized in vascular endothelial cells and are involved in processes such as vasoconstriction, nociception and the physiological regulation of bone function, amongst others. Effects on bone function are important in relation to PCa. Nelson and Carducci (reviewed by Nelson [71]) showed that exogenous ET-1 induces PCa proliferation and enhances the mitogenic effects of insulin-like growth factor (IGF) and epidermal growth factor. Regarding PCa

metastasis in bone, ET-1 production is a major factor in osteoblast overstimulation [72]. PECs produce ET-1, and its receptor, ETa, is present throughout the prostate gland [73–75]. ET-1 is also produced by PCa cells in a bone environment [76]. Experiments using an osteoblast mouse model [72] showed that tumours producing ET-1 (e.g., PCa) act via ETa receptors on osteoblasts to stimulate accelerated bone formation. This abnormal activity is blocked by the ET-1 inhibitor ABT-627 (Atrasentan) [71].

Although ET-1 is important, it is not the only osteoblast stimulator in PCa metastasis. Other factors include up-regulation of the Wnt pathway and production of cytokines, for example, bone morphogenetic protein, TGF- $\beta$ , IGF,

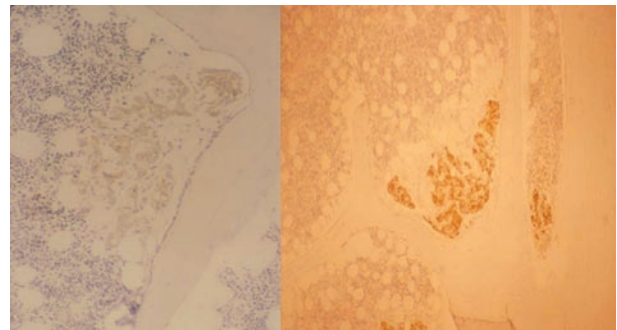


Figure 8. Photomicrographs of two bone biopsies taken from different prostate cancer (PCa) patients. These show the typical appearance of early micro-metastases from PCa. The epithelial cells are stained for prostate-specific antigen (PSA) and appear brown in this image. The cell colonies lie close to the bone surface (uniformly pale), where they stimulate early osteoblastic activity. In the surrounding bone marrow, the fibroblasts are stimulated to induce a desmoplastic reaction. There is no evidence of bone resorption at this stage of the disease process (Reproduced with permission from [2]).



vascular endothelial growth factor, platelet-derived growth factor and MDA-BF [77]. A further interesting aspect of the cytokine balance in PCa metastasis relates to the IGF axis and parathyroid hormone-related protein (PTHrP), which is produced in PCa bone metastases [78]. The prostate-specific antigen (PSA), a known protease, cleaves PTHrP and possibly shifts the balance within the immediate milieu of the prostate metastasis from bone resorption to formation [79, 80]. PSA can also cleave insulin-like growth factor binding protein (IGFBP-3), which in turn increases the levels of IGF-1. This too would have the effect of shifting the axis of stimulation by the metastatic PCa cells towards increased osteoblast activity [81]. Osteoblast hyperactivity is responsible for the measurable increase in bone volume in PCa bone metastases [82, 83] and for the accelerated bone mineralization rate [84]. Prostate tumour-generated bone in these deposits is formed as abnormal 'woven' bone, characteristic of the bone produced in high-turnover states. This is responsible for the sclerotic appearance measured histomorphometrically [83] and seen radiologically in over 90% of patients with advanced metastatic PCa [85].

The traditional view of PCa as osteoblastic obscured for many years the fact that the disease is responsible for major bone destruction. Resorptive effects of PCa were initially suggested following histological studies in bone [86], and the phenomenon was subsequently confirmed after histomorphometric measurements of metastatic bone biopsies [82, 83] and biochemical measurements of bone resorption products in humans [87, 88]. The paradox of increasing bone volume in the presence of bone resorption is explained by histomorphometric studies showing that the resorption of the existing skeleton is accompanied by synchronous replacement of abnormal woven bone, which itself undergoes further resorption [83]. This produces a measurable increase in bone volume coincident with wholesale destruction of the normal skeleton.

Molecular mechanisms responsible for this lytic process arise as the consequences of abnormal concentrations of soluble growth factors produced by the invading PCa, which stimulate abnormal osteoclast activity, inducing bone resorption. Osteoclast recruitment, differentiation and activation by tumours are incompletely understood, but are known to be related to the osteoblast stimulation that results from osteoblastic over-expression of NF- $\kappa$ B (RANK ligand) and the production of osteoprotegerin, known to be increased in PCa metastasis [89]. This effect may also be induced by macrophage colony-stimulating factor, the receptor activator of the RANK ligand and osteoprotegerin [90, 91]. Osteoblasts secrete the RANK ligand, which then induces osteoclast differentiation by binding to the RANK surface receptor on the osteoclast precursor, which in turn stimulates osteoclastogenesis [90]. Osteoprotegerin

plays a key regulatory role in this process by competing for the RANK-binding site on osteoclast precursors. A co-factor in this process is PTHrP. Cancer cells are unable to express the RANK ligand and therefore cannot stimulate osteoclastogenesis by this route. However, when PTHrP is present (as in murine osteoblasts and haemopoietic progenitors in culture [92]), osteoclasts differentiate in the absence of other stimulatory agents, suggesting that PTHrP plays a facilitating role. PTHrP is a major factor in bone resorption in breast cancer [93] and is expressed in both primary tumours and bone metastases of PCa [78].

## 9 Conclusion

The molecular mechanisms of metastasis in PCa are complex and involve a number of specific steps and interrelated mechanisms. A more complete understanding of the molecular mechanisms controlling this process will help to develop novel therapies that may enable us to control this progressively fatal condition.

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