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Dormancy in solid Tumors: Implications for Prostate Cancer

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Abstract

In cancer dormancy, residual tumor cells persist in a patient with no apparent clinical symptoms, only to potentially become clinically relevant at a later date. In prostate cancer (PCa), the primary tumor is often removed and many patients experience a prolonged period (>5 years) with no evidence of disease before recurrence. These characteristics make PCa an excellent candidate for the study of tumor cell dormancy. However, the mechanisms that constitute PCa dormancy have not been clearly defined. Additionally, the definition of tumor cell dormancy varies in the literature. Therefore, we have separated tumor cell dormancy in this review into three categories: (A) Micrometastatic dormancy - A group of tumor cells that cannot increase in number due to a restrictive proliferation/apoptosis equilibrium. (B) Angiogenic dormancy- A Group of tumor cells that cannot expand beyond the formation of a micrometastasis due to a lack of angiogenic potential. (C) Conditional dormancy- An individual cell or a very small number of cells that cannot proliferate without the appropriate cues from the microenvironment, but do not require angiogenesis to do so. This review aims to identify currently known markers, mechanisms and models of tumor dormancy, in particular as they relate to PCa, and highlight current opportunities for advancement in our understanding of clinical cancer dormancy.

Keywords

Dormancy; disseminated tumor cells; prostate cancer; metastasis

Introduction

Although the majority of prostate cancer (PCa) patients are diagnosed and treated by radical prostatectomy (RP), robotic surgery or radiation for the primary tumor, many will experience recurrence of the disease as evidenced by rising PSA levels and subsequently exhibit metastases within an average of 8 years from date of biochemical recurrence (BCR) [1,2]. When considering the entire patient population, including not only those with localized disease, but also those with extracapsular extension, seminal vesicle invasion, and advanced disease, this rate of BCR can be as high as one third of patients. The actual rate of recurrence and recurrence-free survival depends on disease stage. In a recent study of 850 men with mean follow up of 50 months (median 47, range 1 to 192), the estimated 5-year BCR-free survival by negative v. positive surgical margins was 95% v. 83% in pT2, 74% v. 62% in pT3a, and 47% v. 29% in pT3b cases, respectively [3]. Additionally, a study of patients with a PSA failure-free survival rate of 100% at year 5 showed a survival rate of 88% in the same cohort at year 10 [4]. When examining metastasis-free survival, the rate at year 10 was 99.7% for patients with organ confined disease, 95.6% for patients with extracapsular extension and 83.9% for patients with seminal vesicle invasion at time of RP

[4], while higher Gleason scores were also associated with an increased risk of metastases >5 years after RP. These data suggest that the aggressive phenotype of the tumor cells at initial treatment (i.e. high Gleason grade, seminal vesicle invasion and extracapsular extension) is not only an indicator of disease recurrence, but also an indicator of dormancy duration. These data also demonstrate that many patients experience a delay between initial treatment and BCR, which always occurs prior to metastasis. Furthermore, the time from BCR to metastasis is usually greater than 5 years [1,2], making PCa an attractive target for the study of tumor cell dormancy.

While PCa can colonize many distant organs such as the lung, liver and lymph nodes, PCa has a proclivity to metastasize to bone. The consensus is that tumor cells are shed from the primary tumor, initially circulate in the peripheral blood and then extravasate into the bone marrow and other sites. When these tumor cells are present in the peripheral blood, they are referred to as circulating tumor cells (CTC). Once CTC have seeded distant sites, they are referred to as disseminated tumor cells (DTC), and it is these cells that may eventually give rise to demonstrable metastases [5,6]. It is important to note that not all DTC give rise to metastases. Some DTC may die from a failure to adapt to their new environment or from fatal interactions with cells of the immune system. Others may successfully seed and evade immune interactions but fail to actively proliferate, thereby remaining in or entering a state of dormancy. Nevertheless, due to the ease of a blood draw, many groups have focused on the characterization of CTC rather than obtaining a bone marrow aspirate for the study of DTC [7–10].

Circulating tumor cells as prognostic indicators of disease progression and tumor cell dormancy

Numerous groups have identified gene signatures in CTC [7–9,11–14], but these have been of limited prognostic value for recurrence. There are a number of possible explanations for the lack of progress in this area. Firstly, due to the availability of the FDA-approved CellSearch[®] CTC test from Veridex and other, similar methods, clinical efforts have focused on the number of CTC present in a patient's peripheral blood, rather than their molecular analysis. Increased CTC numbers correlate with poor patient outcome [15–17], yet a consensus threshold value for CTC number that signals poor prognosis remains subject to debate [11,18,19]. These studies have found that patient survival based on CTC level was best described as “a continuum function of shedding without a clear threshold” [20], arguing that an individual's change in CTC number may be superior for clinical decision making than a given threshold. Additionally, CTC number is highly dependent on the detection method, further confounding the results obtained at different institutions [21]. Thus, it seems relying solely on CTC number at time of diagnosis or even after therapy most likely will not supply very powerful prognostic information to predict recurrence. The molecular profiles of CTC may provide more prognostic insight.

However, obtaining molecular profiles of shed cells (e.g. CTC) has methodological challenges. For example, CTC are not detectable in the peripheral blood of patients with early stage localized disease [22], thereby limiting their analysis at a molecular level [23]. For patients with metastatic PCa, CTC are readily detectable in the peripheral blood [23], but CTC populations are heterogeneous. This heterogeneity presents a second challenge as some CTC will not successfully extravasate and seed distant sites while others will seed but not readily adapt to their new environment. Both of these challenges confound molecular profiling of CTC and limit the prognostic value of these profiles. In contrast, the interrogation of DTC present in the bone marrow narrows the focus to those shed tumor cells that by either molecular character or random events, have successfully seeded a distant site [24].

Disseminated tumor cells (DTC) as prognostic indicators of disease progression and tumor cell dormancy

While PCa can clinically metastasize to many sites throughout the body (lung, liver, lymph nodes, etc.), it has a particular predilection to metastasize to bone. Whether DTC preferentially localize to bone is unknown, but they are more reliably isolated from bone marrow than from other organ sites. Furthermore, unlike CTC, DTC can be isolated from bone marrow for molecular profiling early in the disease process, and a significant number of patients showing no evidence of disease post initial surgery have detectable DTC in their bone marrow [24]. Furthermore, these DTC are subject to microenvironmental influences on tumor cell behavior. As such, DTC in the bone marrow seem to be of particular value in the study of dormancy in PCa.

Tumor cell homing to the bone marrow microenvironment has been the subject of much research in recent years. Shiozawa and colleagues have shown that in a mouse model of metastasis, human PCa cells from subcutaneous tumors targeted the hematopoietic stem cell (HSC) niche and prevented HSC engraftment. Furthermore, metastasis also positively correlated with niche size [25]. This conforms well to the prevailing belief that metastatic fate is directed by the microenvironment. In fact, TGF- β signaling in the stroma, which is known to be a major regulator in hematopoiesis [26], has been shown to be critical in prostate tumorigenesis and the development of metastases [27].

Nevertheless, the mechanisms of metastasis are not necessarily the same as those involved in the induction and release from dormancy. While the HSC niche may play a role as the site of tumor cell seeding, it is less clear whether the bone marrow microenvironment and hematopoiesis play a significant role in the mechanisms of dormancy. The heterogeneity of primary PCa tumors implies that there may be subpopulations within individual tumors that possess differing metastatic potential. Furthermore, a study of DTC genomic profiles in PCa outlines inherent differences in primary tumors that are carried over into the DTC population present in the bone marrow [28], thus suggesting that the microenvironment may not be the only deciding factor in determining tumor cell fate. The ability to potentially predict the metastatic fate of a patient from DTC molecular profiling make DTC an important clinical target. Molecular profiling of DTC may (1) allow for individualized, more effective treatments (2) potentially reveal targets to prevent recurrence and progression of the disease and (3) avoid “overtreatment” of those patients that have indolent disease. These DTC profiles could potentially shed more light on mechanisms of dormancy and activation than those of the primary tumor. However, before one can begin to use DTC molecular profiles as diagnostic and research tools, we must first define what constitutes a dormant cell.

DTC can be defined as either (A) dormant (not proliferating, no evidence of disease but will eventually recur), (B) slowly growing, (C) senescent (not proliferating and will not recur), or (D) aggressive (actively proliferating without prior growth arrest). With respect to dormancy, there is a clinical need to delineate the non-proliferative states, as they present distinctly different biological processes, which may require different treatment strategies. Tumor cells are thought to undergo senescence as a protection mechanism from aberrant proliferation signals resulting from oncogene activation [29]. Within this context, *Myc* [30,31], *H-ras* [32], ARF/p53 and p16INK4a/pRb [33] activation have all been described to increase β -galactosidase activity, a marker of senescent cells [34]. Unfortunately, senescence and dormancy have often been used interchangeably in the literature to describe non-proliferative tumor cells. Even within the concept of dormancy there is debate as to what mechanisms regulate dormancy or the latency period that signifies dormant disease. In our experience, we have observed patients with no evidence of disease up to and beyond 15

years post radical prostatectomy that have DTC present in the bone marrow and show no clinical evidence of disease.

Why do certain cancers recur after long periods while others remain dormant?

There are a number of possible explanations as to why it could take years for the disease to recur after initial treatment. Cells could be either: (1) slow growing and experiencing a consistent apoptosis/proliferation restrictive loop, (2) lacking angiogenic potential, which restricts their ability to grow beyond a certain size or (3) lacking external cues, either from the microenvironment or other tumor cells, to divide. While it may be difficult to distinguish between these three scenarios given current technology and methods, they nevertheless present distinct biological scenarios with different underlying mechanisms that likely call for different treatment approaches to prevent escape from dormancy. Below we will describe what is currently in the literature in relation to each of these possible explanations for the delayed growth or development of a metastasis.

(1) Slow growth, the apoptosis/proliferation loop and autophagy in DTC

Dormancy may be an equilibrium between proliferation and apoptosis [35], where a constant, low level of proliferation is balanced by apoptosis until the patient recurs. As such, this model of dormancy may be thought of as slow growth. Few models have been proposed in this context of dormancy. Marsden and colleagues have recently introduced a novel model of breast cancer dormancy using primary tumor-initiating cells from patient biopsies [36]. While the model clearly features an initial lack of metastatic outgrowth, it is unclear whether this is due to an equilibrium of proliferation and apoptosis or cell cycle arrest, although the authors define dormancy as the former.

In recent years, there have been markedly more discoveries of micro-metastases and proliferation markers than models relating to slow growth, yet the field is at present less clear about what constitutes a true marker of dormancy versus one of senescence or slow proliferation. This is in part due to the fact that these concepts have not been universally delineated. For example, a study of B-cell lymphoma-2 links maspin to recurrence within 5 years, suggesting maspin may be a marker of slow growth rather than a marker of dormancy [37].

Several markers have been identified in cells undergoing autophagy or slow growth. Autophagy (literally “self-eating”), is the genetically programmed process by which cells degrade cellular proteins and organelles [38]. While this process restricts proliferation, it allows cells to survive adverse conditions such as nutrient starvation and thus could serve as a protection mechanism for DTC encountering non-favorable microenvironments. However, autophagy genes such as Beclin-1, p53 and PTEN are also well-known tumor suppressors [38]. As such, the role of autophagy in tumor progression is likely multi-faceted and requires further study. Nevertheless, breast cancer cells expressing stem cell markers such as aldehyde dehydrogenase I (ALDH1), cluster of differentiation 44 (CD44) and c-jun NH2 terminal kinase have been shown to enter a nonproliferative autophagic state [39], suggesting autophagy may promote survival of cancer cells by allowing them to revert to a stem-cell like state. In fact, hypoactivation of RhoA coupled with an increase in RhoC activity halted proliferation in breast cancer cells, which was reversed by the autophagy inhibitor 3-methyladenine (3-MA) [39], but it is not known whether these cells can regain proliferative potential *in vivo*. Lu and colleagues have argued that elevated expression of the tumor suppressor gene aplasia Ras homolog member I (ARHI) induced autophagic cell death and dormancy in ovarian cancer xenografts [40], yet the observed growth arrest

appeared to be transient, suggesting that autophagic cells may indeed recover to proliferate once more *in vivo*. Still, the transient nature of the growth arrest begs the question whether ARHI truly induced dormancy or whether the results are more consistent with a slow growth model. ARHI has also been demonstrated to regulate senescence [41], highlighting the need to define and delineate the concept and mechanisms of dormancy more clearly. Defective PI3K/Akt signaling and immunosuppression have also been implicated as possible mechanisms of tumor cell dormancy in DTC [42–45], yet it is unclear whether this mechanism induces arrest or slow growth.

(2) Limited angiogenic potential in DTC

Much focus has been lent to the concept of an “angiogenic switch” in tumor dormancy – the notion that tumor cells must recruit and maintain their own blood supply to sustain proliferation. While this is certainly true, it diffuses the concept of dormancy. Metastases are not initiated by tumor masses that require their own vasculature; rather, they initiate from one or a few cells that at some point begin to divide and grow. It is this pre-angiogenic phase that is of particular interest. The need for vasculature arises when cells in the pre-angiogenic phase expand beyond a certain number. Lack of vasculature at this point could induce a secondary growth arrest. In other words, a poorly vascularized, nonproliferative tumor can be thought of as “malnourished” – and whether this constitutes true dormancy or simply slow proliferation/growth-apoptosis equilibrium remains debatable. Nevertheless, the angiogenic switch is an important player in tumor progression and metastasis, and could potentially be involved in mechanisms of release from dormancy, as a clinically significant metastasis requires eventual vascularization. Therefore, it remains important to define why some PCa cells fail to initiate vascularization in the first place (markers of dormant cells) followed by how they overcome this obstacle and eventually allow for vascularization and growth (mechanisms of dormancy release).

Naumov and colleagues have shown that metastases formation is dependent on the angiogenic potential of the metastatic cell, as nonangiogenic subpopulations of various cancers do not form detectable tumors in a subcutaneous mouse model [46]. Furthermore, mouse models of breast adenocarcinoma, glioblastoma, osteosarcoma and liposarcoma show a dormancy period (>120 days) before switching their transcriptome to a rapidly growing, angiogenic phenotype that is retained in subsequent passages [47], suggesting that tumor cells require upregulation of angiogenic factors to initiate growth. However, the subcutaneous models used in these studies do not take into account the microenvironmental influences of the metastatic site (e.g. the bone marrow), and as such, leave some questions as to the physiological relevance of these findings.

Several means have been proposed to turn on the angiogenic switch. It has been suggested that removal of the primary tumor actually promotes metastasis by stimulating angiogenesis and thus stimulating growth in the micrometastasis [48]. Furthermore, epoxyeicosatrienoic acids (EETs), which stimulate angiogenesis, have been shown to promote release from dormancy in liposarcoma and Lewis lung carcinoma [49]. Angiogenic factors have also been shown to increase DII4 expression (a Notch ligand) in endothelial cells, thus stimulating Notch3 signaling and promoting growth in colorectal cancer cells and T-cell acute lymphoblastic leukemia [50]. However, these studies all reported growth arrest of micrometastases or a large number of injected cells, not individual cells. In fact, when examining growth of a small number of cells (akin to what likely occurs in clinical metastasis), angiogenic factors may not play a role in release from growth arrest [49]. Since it is likely that individual cells metastasize from the primary tumor, it becomes apparent that there is a need to distinguish mechanisms of dormancy for micrometastases from those of individual DTC.

(3) Conditional dormancy in DTC

While a micrometastasis will eventually reach a critical size that requires angiogenesis for continued proliferation, a single cell has no such requirements and thus its growth arrest must be initiated by alternative means, such as an arrest in the G₀/G₁ cell cycle transition [51]. There have been some discoveries with respect to tumor cell markers associated with G₀/G₁ cell cycle arrest, but a consensus marker remains elusive. High Bmi-1 expression in primary tumors has been associated with breast cancer relapse after 10 years, while lower expression of Bmi-1 was associated with faster recurrence [52]. This suggests that Bmi-1 may be a marker of dormant cells, though it is unknown whether those patients with high expressing Bmi-1 tumor cells experience a loss of Bmi-1 upon recurrence. Interestingly, expression of cytokeratin 19 (CK19), which is commonly employed in the isolation of breast cancer DTC, is associated with cell cycle arrest, reduced cell motility and increased drug resistance [53]. The urokinase plasminogen activator receptor (uPAR) has also been identified as a player in tumor dormancy and progression, as it regulates invasion, intravasation and metastasis [54]. Perhaps most interestingly with respect to this study, uPAR was implicated in the switch between single cell tumor dormancy and proliferation. Specifically, low uPAR expressing HEP3 cells showed an overall decrease in proliferation not attributed to apoptosis, proteolysis or poor vasculature [55]. Low levels of uPAR subsequently resulted in decreased association with $\alpha_5\beta_1$ integrin, deactivation of the Ras-ERK pathway, downstream activation of p38^{SAPK} and ultimately G₀/G₁ arrest in breast cancer, melanoma, PCa and fibrosarcoma cell lines [56–58].

While markers of dormancy could have great prognostic value, the ultimate goal is to target DTC such that they remain dormant. To do so, we must not only identify DTC by biomarkers, but also understand the mechanisms by which the DTC enter into and exit from dormancy. While the direct study of DTC with regards to these mechanisms is in its infancy, several such mechanisms have been proposed to induce or release cancer cells from dormancy in numerous *in vitro* and *in vivo* proliferation models. Barkan and colleagues argue that cues from the tumor microenvironment, in particular signaling through α_1 integrin, are key regulators of dormancy in prostate, breast and renal tumors. Specifically, a blockade of α_1 signaling has been shown to induce a dormant state and maintain cell cycle arrest in a 3D *in vitro* model [59]. The extracellular matrix (ECM) has been shown to be of critical importance in inducing and releasing cells from cell cycle arrest. Cytoskeletal reorganization and formation of stress fibers as a result of ECM signaling through integrin α_1 has been shown to induce downstream phosphorylation of myosin light chain (MLC) by its kinase (MLCK), thus resulting in proliferation [59]. Nevertheless, it is as of yet unclear which conditions result in ECM activation or shutdown of integrin α_1 signaling. White and colleagues observed that in a mouse model of α_1 integrin disruption, breast cancer cells showed no signs of proliferation and remained single cell entities [60]. Specifically, phosphorylation of FAK, which has been implicated in cell cycle progression [61], was absent in cells lacking α_1 integrin, suggesting that the observed non-proliferating cells may be dormant. Moreover, a mouse model of pancreatic cancer showed that loss of α_1 integrin induces tumor cell dissemination into the lymphatic system, prevented metastasis formation and reduced both tumor cell proliferation and G₀/G₁ cell cycle arrest [62].

The microenvironment has also been implicated in dormancy induction via the transfer of microRNAs (miRNAs) targeting CXCL12 from the stroma to breast cancer cells via gap junctions [63]. Nuclear pore architecture may also play a role in dormancy induction, as knockdown of nucleoporin 62 (NUP62) has been shown to cause cell cycle arrest in ovarian carcinoma [64]. Taken together, these studies suggest that the microenvironment and cellular architecture are key regulators of dormancy prior to any type of angiogenic restriction, though it remains to be seen if the same mechanisms described herein regulate dormancy in PCa DTC.

So what is tumor cell dormancy and how do we target it?

While it appears that metastatic outgrowth is regulated by a balance of apoptosis and proliferation, as well as the ability of the tumor to maintain a blood supply, the literature points to a fundamental event prior to the need for any type of vasculature that determines whether a DTC will initiate proliferation or not. Thus, we can think of dormancy as consisting of a pre-angiogenic phase, in which individual cells have the potential but lack the ability to proliferate; and a post-angiogenic phase, where a larger number of cells depend on nutrient delivery in order to continue proliferating (Figure 1). With respect to this distinction, we know that tumor growth requires vascular proximity of 0.2mm or less [65], yet we do not know the threshold size for pre-angiogenic proliferation. The involvement of factors such as integrin 1, FAK, and Ras in cell cycle regulation would suggest that motility and adhesion play an integral role in dormancy, yet little is currently understood about what induces release from the pre-angiogenic dormant phase, particularly in PCa. Furthermore, the current debate over what constitutes a dormant cell presents a challenge to understanding the progression from dormancy to the pre-angiogenic and post-angiogenic phase.

A significant body of evidence suggests that chemokines and cytokines released from the microenvironment play a critical role in the induction or release from dormancy [25,27,60,63]. VCAM-1 has been found to be of importance in the formation of breast cancer (BrCa) bone metastatic lesions. Specifically, VCAM-1 expression promoted bone-metastatic outgrowth in BrCa sublines that originally displayed a latency period in bone [66]. Interestingly, VCAM-1 expression did not promote the same metastatic outgrowth in the parental line, suggesting that dormant cells acquire VCAM-1 expression as a means of escaping dormancy. This effect appeared to be dependent on the bone microenvironment, as VCAM-1 expression did not affect proliferation rates *in vitro*. Indeed, VCAM-1-expressing BrCa cells had the ability to recruit and immobilize pre-osteoclasts to establish an osteoclastic niche [66]. Since bone is a nutrient rich “soil”, increased localized resorption as a result of recruited osteoclasts likely releases a variety of growth factors and cytokines that promote metastatic outgrowth.

This mechanism likely holds implications for PCa. Even though PCa metastases in bone are primarily osteoblastic, we have observed a varying range of osteolytic activity in almost all PCa bone metastases samples obtained from rapid autopsy [67]. Furthermore, osteoclast and osteoblast activity are intimately linked via RANK-L [68]. Thus, increased osteoclast activity could likely spur activation and proliferation of osteoblasts through release of growth factors from bone, accounting for the ultimately osteoblastic nature of PCa bone metastases.

In addition to osteoclasts, chemokine recruitment of bone marrow stromal cells has also been implicated in the activation of indolent tumor cells. Osteopontin secreted by aggressive tumors was shown to activate and mobilize stromal cells to spur the growth of indolent tumors [69]. This activating effect persisted even after the removal of the primary tumor, suggesting that primary tumor osteopontin expression systemically alters stromal cells early in the disease process to facilitate late disease recurrence. This mechanism has significant implications for PCa dormancy, suggesting that the primary tumor could systemically affect stromal cells to subsequently activate indolent DTC even after the primary tumor has been removed.

Stress may also be implicated in the release of factors from the microenvironment that spur PCa growth. Genotoxic damage in fibroblasts caused by chemotherapeutics increased secretion of MMPs and chemokines, including WNT16B, p16, p21 and IL8 [70].

Specifically, WNT16B was shown to signal through β -catenin to induce EMT and promote PCa growth. This upregulation of WNT16B was mediated by NF- κ B, which is known to be involved in stress-associated induction of inflammatory networks like secretion of IL-6 and IL-8. Of these, IL-6 has been implicated in osteoclast activity, which could very well result in the release of growth stimulatory cytokines from the bone matrix and promote disease recurrence by creating a permissive environment for tumor growth. While patients experiencing PCa disease recurrence may not necessarily be receiving chemotherapy, the inflammatory mechanisms activated by genotoxic damage are some of the same mechanisms activated by stress, thus implicating stress as a potential factor in PCa disease recurrence.

It is also important to note that DTC isolated early in the PCa disease process appear to retain the molecular profile of the primary tumor [28,71], begging the question whether it is the inherent nature of the DTC or the microenvironment that directs dormancy fate. We hypothesize that both the molecular profile of the DTC and its microenvironment are critical in regulating proliferation, though dormancy may be regulated by a specific subset of these conditions. To elaborate, a cell possessing a non-proliferative molecular profile will likely never proliferate, whether it resides in a favorable microenvironment or not. However, since these cells do not have the ability to eventually proliferate, they cannot be classified as dormant, but are likely senescent or apoptotic. Conversely, a cell with a profile favorable for proliferation that encounters an unfavorable microenvironment will likely enter one of the dormancy states outlined above. Lastly, should this cell encounter a favorable microenvironment, it will likely enter an aggressive state without a dormancy period. Until we define such conditions and molecular profiles, we can only hypothesize on the fate of DTC.

As such, there is a clear and present need to further study pre-angiogenic events that lead to proliferation of disseminated cells. While there has been some progress in this study in recent years, particularly in breast cancer [36,37,52,53,60], there has been little progress in understanding PCa dormancy. This is particularly disconcerting as an extended dormancy period is observed in many PCa patients, and the recurrent disease often becomes castration resistant, for which there are limited treatment opportunities. The technical challenges of interrogating small numbers of cells, lack of dormancy models, acquiring samples of dormant cells and the heterogeneity of PCa have restricted progress in the past. With the advent and characterization of numerous PCa xenograft models, we now have the capability to generate physiologically relevant *in vivo* models of PCa to ultimately develop models of PCa dormancy [72]. Furthermore, using established methods to isolate and interrogate DTC [24,28], we now refined these techniques to analyze single cells (Morrissey et al., in submission). Therefore, we can now attempt to address the challenge of PCa heterogeneity. Taken together, these advances now provide the opportunity to define mechanisms of *in vivo* dormancy, leading to the discovery of biomarkers to predict outcome and development of targeted therapies to prevent disease recurrence.

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Abbreviations

CRPC	castration resistant prostate cancer
CTC	circulating tumor cell

DTC	disseminated tumor cells
PCa	prostate cancer
PSA	prostate specific antigen

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Fig. 1. Fate of Disseminated Tumor Cells

Tumor cells in circulation either disseminate as a single cell or group of cells. Single cells with defective integrin signaling likely enter cell cycle arrest and become dormant, while those with active cell signaling begin to proliferate and form a group of cells. At this juncture, cells can once again experience a nonproliferative state, either through proliferation and apoptosis equilibrium or a lack of angiogenic potential, both of which are probably mediated by unfavorable microenvironmental conditions. If the surrounding microenvironment presents favorable conditions that allow for continued growth and angiogenesis, the small cluster of cells then continues to proliferate to form an active metastasis.

Fig. 2. Activation of Disseminated Tumor Cells

Disseminated dormant cells can be induced to proliferate by the release of various growth factors, either from the bone matrix itself or cells in the bone microenvironment. This release of growth factors can be brought about in several ways: Stress, which activates inflammatory mechanisms in the tumor cells, can bring about the release of VCAM-1 to recruit osteoclasts and release growth factors from the bone matrix. Similarly, inflammatory mechanisms can also promote the release of growth factors by fibroblasts. Lastly, high osteopontin expression, which may be inherent or acquired at the metastatic site, could induce the recruitment of stromal cells to facilitate eventual growth of tumor cells.