

Revisiting Seed and Soil: Examining the Primary Tumor and Cancer Cell Foraging in Metastasis

Amber E. de Groot¹, Sounak Roy¹, Joel S. Brown^{2,3}, Kenneth J. Pienta¹, and Sarah R. Amend¹



Abstract

Metastasis is the consequence of a cancer cell that disperses from the primary tumor, travels throughout the body, and invades and colonizes a distant site. On the basis of Paget's 1889 hypothesis, the majority of modern metastasis research focuses on the properties of the metastatic "seed and soil," but the implications of the primary tumor "soil" have been largely neglected. The rare lethal metastatic "seed" arises as a result of the selective pressures in the primary tumor. Optimal foraging theory describes how cancer cells adopt a mobile foraging strategy to balance predation risk and resource reward. Further selection in the dispersal corri-

dors leading out of the primary tumor enhances the adaptive profile of the potentially metastatic cell. This review focuses on the selective pressures of the primary tumor "soil" that generate lethal metastatic "seeds" which is essential to understanding this critical component of prostate cancer metastasis.

Implication: Elucidating the selective pressures of the primary tumor "soil" that generate lethal metastatic "seeds" is essential to understand how and why metastasis occurs in prostate cancer. *Mol Cancer Res*; 15(4): 361–70. ©2017 AACR.

Introduction

Metastatic prostate cancer is responsible for approximately 26,000 deaths per year in the United States. Despite clinical advances improving survival of men with localized prostate cancer, metastatic disease remains incurable (1, 2). Prostate cancer nonrandomly metastasizes to the bone, resulting in high patient morbidity and mortality (3). Over the last several decades, models have emerged to describe the general sequential steps of the metastatic process: detachment from the basement membrane, local invasion, intravasation into the vasculature, systemic dissemination, cellular extravasation, and metastatic colonization (Fig. 1; refs. 4, 5). The research investigating the selective colonization of the bone is largely based on Paget's "seed and soil" hypothesis that suggests that metastatic cancer cell "seeds" must fall on congenial target organ "soil" (6–9). While the compatibility between a metastatic prostate cancer cell and the bone metastatic site has been extensively described, little work has investigated the relationship of the premetastatic primary prostate cancer cell "seed" and the site of origin "soil."

Applying evolutionary ecology principles to the study of cancer has provided a deeper understanding of the selective pressures that give rise to the eventually successful metastatic seed (10–19).

Previous work has demonstrated that cancer cells move within the primary tumor and that there is variability in movement patterns among individual cells (14, 20–22). The cells that readily move in the primary tumor may be predisposed to disseminate, underscoring the importance of uncovering the origins of cell motility within a tumor. Despite the advancements in understanding the mechanisms of cell movement (20–22), the determinants of cell movement remain unclear. Optimal foraging theory (OFT), a subdiscipline of evolutionary ecology, can be used to describe and predict movement in a heterogeneous environment and may be applied to prostate cancer to elucidate the influences of cell movement within a tumor.

Studying cancer as an invasive species provides insight into the necessary phenotypic characteristics of the metastatic "seed" and how those traits are selected for. To disseminate to a distant secondary habitat, invasive species utilize established dispersal corridors, regions of uninhabitable geography linking two distant favorable habitats. Metastatic prostate cancer cells emigrate from the primary tumor via distinct corridors: blood vessels, lymphatics, and nerves (Fig. 4; refs. 23–29). Understanding the selective pressures that promote or inhibit a cell's entry into these corridors will provide a better understanding of the requirements for a successful metastatic "seed." These ecological principles shed light on how the primary tumor "soil" and metastatic routes select for successful metastatic cells.

Optimal foraging of prostate cancer

One of the universal properties of life, as an animal, a plant, or a cell, is the need for acquiring resources to fulfill the basic metabolic needs to support life. Resources are the consumable and depletable factors essential for the survival, proliferation, and movement of an individual (Table 1). To find and consume these resources, an organism must forage by employing different strategies dependent on the balance of risk, reward, and ability (Fig. 2). OFT states that the optimal foraging strategy for a particular organism is one that provides maximal resources at minimal

¹The James Buchanan Brady Urological Institute at the Johns Hopkins University School of Medicine, Baltimore, Maryland. ²Department of Biological Sciences and UIC Cancer Center, University of Illinois at Chicago, Chicago, Illinois. ³Integrated Mathematical Oncology, Moffitt Cancer Center, Tampa, Florida.

A.E. de Groot and S. Roy contributed equally to this article.

Corresponding Author: Sarah R. Amend, The James Buchanan Brady Urological Institute at the Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Marburg 113, Baltimore, MD 21287. Phone: 410-504-4974; Fax: 410-955-0833; E-mail: samend2@jhmi.edu

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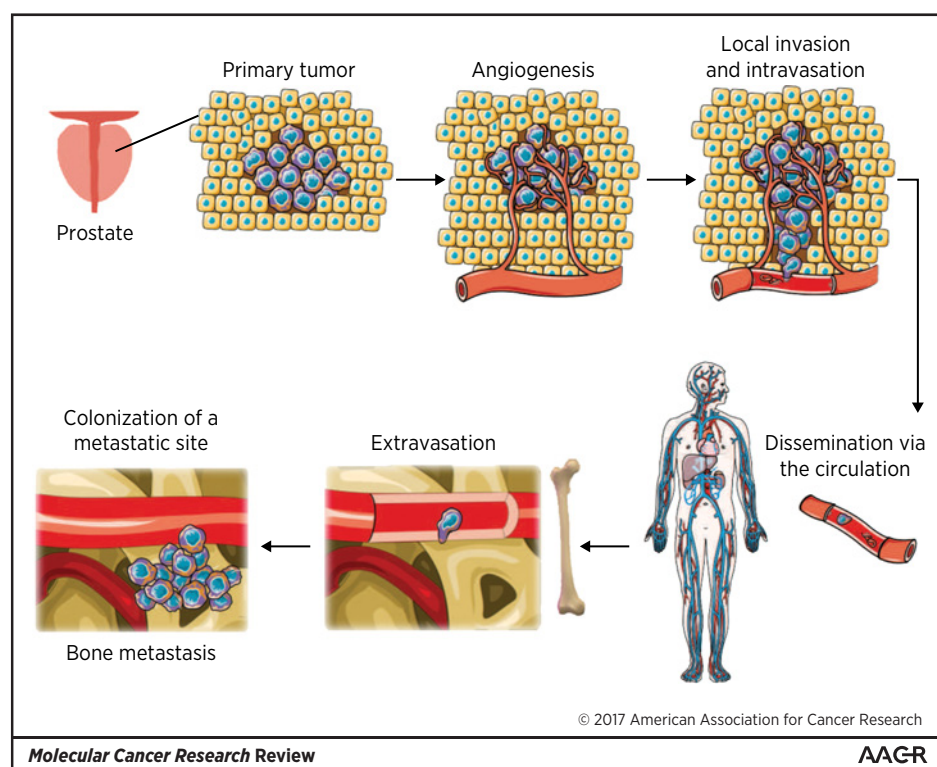


Figure 1. Steps of the metastatic process. Metastasis is characterized by a series of sequential steps: primary tumor formation, recruitment of blood vessels through angiogenesis, cancer cell invasion of local tissue, and entry into dispersal corridors such as blood vessels. Disseminated cells travel through the circulation and upon reaching a suitable secondary site such as the bone, extravasate from the blood vessels, and colonize to form bone metastases (modified from Servier Medical Art by Servier licensed under CC BY 3.0.).

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cost to the individual (30, 31). Optimal foraging strategies vary among members of a species and depend on the state of the individual as well as the properties of its environment (31). Foraging strategies are described broadly as a combination of stationary versus mobile techniques. Stationary foragers remain in one place and wait for local resources to replenish. In contrast, mobile foragers optimize foraging potential by moving among resource patches as they become depleted over time. Organisms will adapt their foraging strategies based on the immediate characteristics of their habitat, and the fittest indi-

viduals will have adopted the most successful or optimal foraging strategies. In this way, OFT describes when and how an organism should move through its environment so as to balance the benefits and costs of foraging.

Cancer cells are generally stationary foragers that focus their energetic efforts on proliferation rather than movement (32). A select few, likely those that undergo an epithelial-mesenchymal transition (EMT), will have the option to employ a mobile foraging strategy. Generally these cells have higher energy requirements and consume more resources than their stationary

Table 1. Definitions of prostate cancer optimal foraging theory and dispersal

Term	Definition	Examples	
		Ecology	Prostate cancer biology
Resource	A depletable factor essential for survival, movement, or proliferation	Nuts, water, oxygen	Sugars, lipids, oxygen, etc.
Patch	A depletable area of localized resource	Oak tree	Region adjacent to a blood vessel
Habitat	The physical abiotic region in which an individual resides, segmented into resource patches	Forest	Prostate tumor
Species	A group of individuals with a shared lineage and similar functional traits	Squirrels, hawks	T-cells, prostate cells, fibroblasts
Individual	A species member that functions independently and consumes resources	Squirrel	Cell
Foraging	The search for and consumption of resources in a habitat (mobile or stationary strategy)	Squirrel forages for nuts	Cancer cell forages for oxygen
Stationary forager	An individual that forages without moving to another patch regardless of patch depletion	Sponge	Epithelial cell
Mobile forager	An individual that forages by moving among patches	Squirrel, hawk	Mesenchymal cell
Predator	An individual that attacks and kills another individual	Hawk	T-cell, M1 macrophage
Dispersal corridor	An inhabitable path through an inhospitable region linking two or more favorable habitats	Railroad tracks, river	Blood vessel, lymph vessel, nerve
Adaptation	A fitness-enhancing phenotypic trait that is selected for by an external selective pressure	White fur color of a snowshoe hare	Resistance to anoikis by a circulating tumor cell

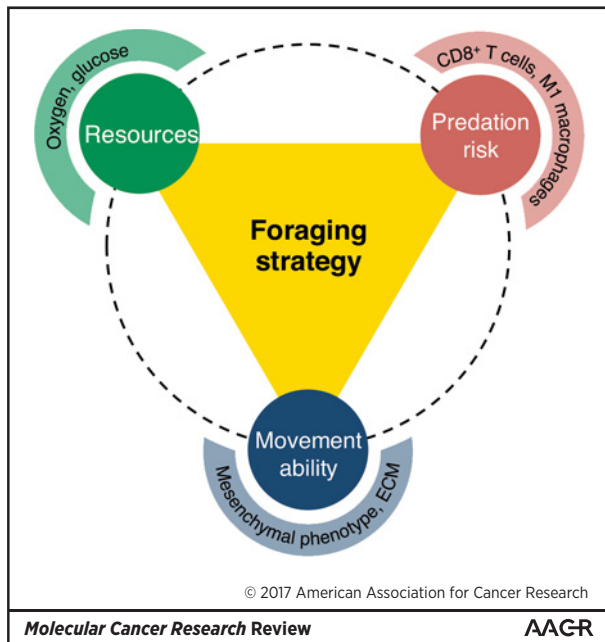


Figure 2.

Optimal foraging of prostate cancer cells. The optimal foraging strategy employed by a cancer cell is influenced by three interacting factors: available resources (e.g., oxygen, glucose), predation risk (e.g., cytotoxic T cells, M1 macrophages), and movement ability (e.g., mesenchymal phenotype, permissible ECM).

epithelial-like counterparts (33). As very few cells in the primary tumor undergo EMT, the number of stationary foraging cells is likely to vastly outnumber mobile foraging cells.

Applying OFT to cancer biology introduces the novel concept of cancer cell foraging in which a cancer cell's potential to disseminate from the primary tumor is influenced by its foraging strategy. The optimal foraging strategy of an individual is influenced by movement ability, resource distribution, and predation risk (Fig. 2). These same influences dictate cancer cell foraging behavior. While the primary tumor habitat promotes both foraging strategies, the few cells that adopt mobile foraging are more likely to possess the traits necessary for successful metastasis.

Movement ability is essential for mobile foraging of premetastatic prostate cancer cells

The ability of an organism to move through space is determined both by the organism's intrinsic capacity to move as well as the environmental constraints on movement. For example, a sea sponge is obligatorily stationary because it possesses little capacity to move. In contrast, a squirrel in a forest has the capability to act as a mobile forager by transporting itself from tree to tree in search of resources. However, if confined by a cage, the squirrel can no longer act as a mobile forager because its environment does not permit movement, forcing it to adopt a stationary foraging strategy. Thus, characteristics of both the individual and the environment limit whether an individual can incorporate movement into its foraging strategy.

These same physical limits constrain cancer cells in the primary tumor: the cell must have the capacity to move and the tumor environment must permit movement. The genetic and molecular

bases for cell movement behaviors and metastatic propensity in prostate cancer have been extensively studied (21, 33–35). Cell tracking experiments reveal that mesenchymal prostate cancer cells exhibit increased general nondirected movement compared with epithelial cells derived from the same parent prostate cancer cell line *in vitro* (33). This increased movement phenotype is a direct product of the cell's intrinsic properties, predisposing it to a certain behavior.

In addition to a cell's inherent capacity to move, the tumor environment must permit cell movement. Many physical properties of the tumor including extracellular matrix (ECM) organization, pH, and interstitial fluid pressure influence tumor cell dissemination (21, 22, 36, 37). Variance in these physical conditions may determine whether the environment is conducive for cell movement. For example, the ECM may facilitate cell motility by providing a stiff substrate for cellular focal adhesion necessary for cell movement (38). Conversely, the ECM structure and organization can inhibit movement depending on characteristics such as fiber composition and alignment (39). As a classic example, the basement lamina confines benign cells to the gland lumen (40).

In addition to the physical properties of the tumor, other environmental factors, such as other cell species within the tumor, make the environment more or less permissible to movement. For example, cancer-associated fibroblasts and M2-like tumor-associated macrophages secrete enzymes that remodel the ECM thereby increasing cancer cell movement opportunities by altering the physical scaffolding of the environment (41).

In OFT, the environment characteristics coupled with cellular movement phenotype determines the cell's ability to incorporate movement into its foraging strategy. While an individual's capacity for movement determines its ability to adopt a mobile foraging strategy, it does not mean that the cells will employ the option of mobile foraging. Movement through a heterogeneous environment is associated with certain risks (i.e., predation) and rewards (i.e., resources). The optimal foraging behavior of a cell will not only depend on its ability to move but also the predation risks and resource rewards associated with mobile foraging behaviors (Fig. 2).

Resources are distributed heterogeneously into patches

A major determinate of an individual's foraging strategy is the availability of resources and their distribution throughout the habitat. Resources include all of the depletable factors consumed for survival, proliferation, and movement (Table 1). Resources are often distributed heterogeneously throughout a habitat. For example, acorns are necessarily concentrated on their parental tree or on the ground nearby, but are scarce in the adjacent space. Therefore, a foraging squirrel's encounter rate with acorns increases as it approaches the tree. OFT defines these discrete areas of localized resource as "patches" (Table 1; Fig. 3B). Because patches are distributed nonhomogeneously both in geographical space and in time, as resources are consumed by all members of the community, patchy habitats promote movement throughout a region as an optimal foraging strategy.

A similar pattern of patchy resource distribution has been observed in tumor habitats (Fig. 3A; refs. 42–46). In the case of cancer cells, while the complete repertoire of resources has not been defined, resources likely include oxygen (47), carbon and nitrogen sources (sugars, amino acids, and lipids; ref. 48), and metal ions (49). These resources are supplied to the tumor by the

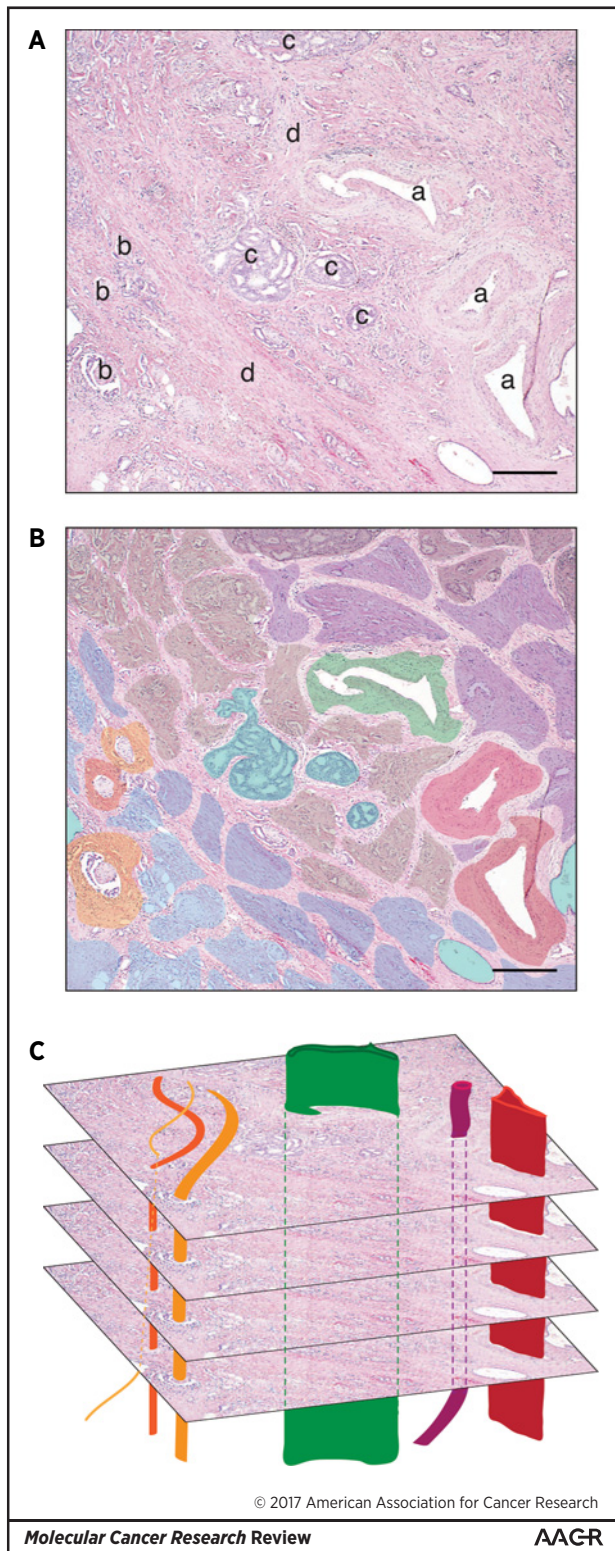


Figure 3. Prostate cancer resource patches and dispersal corridors. **A**, Primary prostate tumor from prostate cancer patient radical prostatectomy (a, lymphovascular vessel; b, nerve; c, intraductal carcinoma; d, stromal infiltration). **B**, Prostate cancer resource patches: colored regions represent patches within the

local ECM environment, cell debris, and tributary-like influxes from nerves and the blood and lymph vasculatures. These resource suppliers are distributed heterogeneously throughout the tumor (39, 43, 50) providing different areas of the tumor habitat with different resource levels and types (Fig. 3B).

Patches with higher levels of resource will take longer to deplete, allowing the organism increased resource consumption. Once a patch is depleted, stationary foragers will reduce their metabolic needs to stay in place and wait for resources in the patch to replenish. Under the same pressure of resource decline, mobile foragers will desert the depleted patch and physically move in search of for a more favorable resource patch elsewhere (assuming no confounding factors such as increased risk of predation).

In addition to resource abundance, however, the variety of resource types in a given patch also influences foraging strategy. As each organism requires a variety of resources to fulfill a variety of energy requirements, an organism's time in a patch also depends on the types of resources the patch contains. For example, a squirrel requires both a source of carbohydrates, such as nuts, and a source of water. A patch that offers nuts but no available water will be less valuable and abandoned earlier than a patch that offers nuts and readily available water (51). Thus, an organism's response to the amount, type, and distribution of resource contributes to its foraging strategy and movement behavior throughout a habitat.

This OFT concept of patches has been applied to cancer to explain and model the distribution and abundance of varying resource types throughout the tumor (42). As with ecology, the resources in a patch diminish and the foraging individual must search for more. Overcrowding, such as when a large number of cancer cells deplete the oxygen supply and create regions of hypoxia, may accelerate resource depletion (10, 46). To search for resource, the majority of cells remain in the same location to wait for local resource to replenish, but a select few invade through or out of the local tumor space in pursuit of more advantageous resource patches. The latter technique has been observed in cell lines with oxygen gradients in three dimensional matrix models *in vitro* (46). As with ecology, available resource abundance and variety affect whether an individual adopts a mobile foraging strategy: a patch with high levels of sugars will likely take longer to diminish and require less movement to optimally forage. However, if the patch lacks an essential resource, such as oxygen, then an optimal foraging strategy would include movement out of that patch after a shorter amount of time. Thus, cell movement throughout the primary tumor in the context of OFT is in large part a response to resource distribution in the primary tumor.

Predation risk alters foraging strategy

Foraging strategies of virtually all organisms are strongly impacted by the predation risk-associated exploiting or moving between resource patches (52). Predation risk describes the likelihood of being attacked by a predator as determined by the

primary tumor and depict spatial heterogeneity at single moment in time. Variations in color represent variations in patch characteristics (i.e., resource and predation risk). Importantly, although not depicted, patch geography and characteristics change over time. **C**, Dispersal corridors including blood vessels (red and maroon), lymph vessels (green), and nerves (orange) intersect primary tumor patches and provide a route for long-distance dissemination out of the primary tumor habitat. (H&E; scale bar, 300 μ m; image courtesy of Dr. Tamara Lotan, Johns Hopkins University)

number of predators nearby, the predator's lethality if encountered, and the prey's ability to escape or evade detection. An organism's ability to camouflage can reduce predation risk even in areas with large numbers of predators by reducing the likelihood of detection and attack. However, when the organism is eventually detected, it must evade the predator's attack to survive. Evasion can take the form of moving away from areas of high predation risk (31). An ecological example of managing predation risk is observed in rabbit phenotypes and behavior. In the winter, snowshoe hares molt from brown to white fur. The color change serves them well in the snow that inevitably occurs in the boreal forests of Canada. In addition to camouflage, hares also have their rapid hopping gait as a means for evading attack by a predator such as a lynx.

In the prostate cancer setting, predators include the antitumor immune cells such as CD8⁺ cytotoxic T cells and M1 macrophages that seek out, destroy, and consume their prey: the cancer cell (Table 1; refs. 53, 54). As such, a risky patch for the tumor cell will have higher CD8⁺ T-cell and M1 macrophage infiltrate. These immune cell predators are heterogeneously dispersed throughout the tumor (55), presumably with higher concentrations near the blood and lymph vessels where they enter the tumor habitat. Therefore, predation risk likely increases with proximity to the resource-high lymphovascularity with additional risk as they enter lymph or blood vessels. Cancer cells decrease their predation risk by expressing programmed death ligand 1 (PD-L1), which camouflages them from these predators by inhibiting CD8⁺ T-cell proliferation and cytokine secretion (56). PD-L1-expressing cells may be able to forage successfully and more thoroughly in the high resource patches located closer to the vasculature regardless of the increased immune cell presence. In addition to camouflage, the optimal foraging strategy of cancer cells in response to predation risk will include increased movement to evade attack and move away from high concentrations of immune cells. Therefore, a cancer cell's tactic for immune evasion is part of its foraging strategy and helps explain its movement behavior within the tumor.

Adaptive phenotypic traits selected for in the primary tumor: stationary versus mobile foragers

Movement ability, resources, and predation risk each contribute to determining an individual's foraging strategy thereby explaining the primary tumor influences on cancer cell behavior (Fig. 2). Applying OFT to prostate cancer reveals that a variety of foraging strategies are adopted by cancer cells, which creates phenotypic heterogeneity within the tumor. The most successful of these strategies are selected within the primary tumor. While a stationary foraging is a common successful strategy among primary tumor cells, some cells adopt mobile strategies with behavior similar to invasive species. The adaptations of mobile foragers, such as the ability to move or evade predation, likely increase their ability for metastatic behaviors such as intravasation or survival in the high-predation circulation. Because of these adaptations, mobile foragers are more suited for successful dissemination from the primary tumor and potential metastasis. Clinical evidence for the selection of mobile foragers by primary tumor conditions includes the positive correlation between hypoxic primary prostate tumors and biochemical recurrence (47). This study exemplifies how cell adaptations resulting from low-resource primary tumor conditions may promote metastatic capability.

Tributary-proximal patches positively select for potential disseminating cells

The richest resource patches in the primary tumor are located in close proximity or adjacent to the blood vessel, lymph vessel, and nerve tributaries that provide a constant supply of high levels of resources (Fig. 3C). Delivered resources include oxygen and sugars supplied by the blood and nerve growth factor and other neurotrophins supplied by nerves (57). In addition to providing high-resource patches, as source of entry for host immune cells these tributary-proximal patches are also characterized by high inherent predation risk. Cancer cells are attracted to high resource areas and therefore attracted to these patches. The cells that move into and remain in these patches must also have the ability to evade predation, either through camouflage (e.g., PD-L1 expression) and/or high movement ability. The same phenotypic traits selected for in the tributary-proximal mobile foragers are also favorable for successful metastasizing cells: preference for high cell movement, ability to invade the local tumor space, and ability to sense and evade predation by immune cells. These traits not only increase fitness within tributary-proximal patches but also confer metastatic potential. In this way, tributary-proximal patches positively select for cells predisposed to metastatic behavior.

Each of the three resource tributary types also acts as a metastatic route (23–25). As a cell moves into patches near these routes, a cell's likelihood of entering the route and dispersing from the primary tumor increases. By exhibiting traits suited for metastasis and by increasing their encounters with metastatic routes, mobile foragers increase their potential for dissemination and metastasis. Evolution within the tumor unwittingly selects for cancer cells capable of metastasizing and selects for movement and patch use behaviors that place a mobile foraging cancer cell type near blood, lymph, and nerve routes of dispersal.

Dispersal corridors are barriers to dissemination

Prostate cancer acts as an invasive species as cancer cells leave their native primary tumor to establish colonies in distant secondary sites, most commonly in bone (58). In order for an invasive individual to colonize a distant site, it must first escape its native habitat. In ecology, invasive species often escape via dispersal corridors, pathways that connect two or more distant regions (Table 1). In general, dispersal corridors themselves cannot sustain the population either because of limited resource availability or high predation risk. They do, however, provide a low level of resources and relative safety from the surrounding hostile environment. A common ecological corridor is a railroad track that is used by animals to travel through inhospitable urban habitats. While the tracks do not provide the essential requirements of a coyote habitat (ample resources, shelter, etc.), the coyote may move between suitable habitats without encountering the extreme and unfamiliar predation risk of a city (59).

In addition to providing a relatively safe and unhindered passage across hostile landscape between favorable habitats, a dispersal corridor also acts as a selection barrier. The dispersal corridor environment may include unfamiliar physical conditions, varied predation risk, and different resource levels than the primary habitat (60). Organisms utilizing the corridor must possess the necessary characteristics for entry into and survival within this unfamiliar environment. Therefore, while these selective corridors allow for rapid and long-distance expansion of a subset of potential invaders, they simultaneously prevent the spread of the organisms lacking the characteristics for corridor

entry and survival. Thus, dispersal is limited to individuals with particular traits.

Invasive prostate cancer cells escape the primary tumor and metastasize via three dispersal corridors: hematogeneous spread via blood vessels, lymphatic spread via lymph vessels, and perineural invasion (PNI) via nerves (Fig. 3C; refs. 23–29). Similar to ecological dispersal corridors, these routes of dissemination function as filters between two distinct habitats, only permitting cells with particular phenotypes to utilize the corridors and potentially establish clinical metastases while confining others cells to the primary tumor (11, 61). The selective properties of dispersal corridors are 2-fold: barriers to entry into the corridor and barriers to long-distance dispersal once in the corridor.

Selection pressures of entry into dispersal corridors

The entry barriers faced when entering the dispersal corridor are determined by the corridor's characteristics. For example, a river with thick underbrush and ground cover on its banks will have a high barrier to entry. Only animals that physically break through the underbrush will be able to enter the dispersal corridor. Thus, the corridor exerts selective pressure for certain characteristics and only organisms with the appropriate characteristics will have the opportunity to attempt dispersal along that corridor. Importantly, however, barriers to entry are context-dependent: different organisms with varying adaptations will be able to invade the corridor depending on the selective properties of the barrier.

In a primary prostate cancer tumor, there are different barriers to entry depending on the characteristics of the dispersal corridor. Blood and lymph vessels are corridors with constant one-dimensional fluid currents analogous to a river. When entering a blood or lymph vessel, a primary tumor cell faces physical barriers such as the endothelial cell lining and surrounding basal lamina. Cells enter these vessels by two mechanisms: active intravasation or passive sloughing. Passive sloughing is most likely when entry barriers are low as typically observed in vessels with permeable basal lamina and endothelial cell layers (62). Vessels that allow entry by passive sloughing select for a wide range of cells that are capable of detachment by an external force such as a current. This cell population includes both mobile (mesenchymal) and stationary (epithelial) foragers.

Vessels that require entry by active intravasation have less permeable basal lamina and endothelial cell layers (62) and select for mobile foragers with high capacity for locomotion and invasion (32). Invasive prostate cancer cells overcome the ECM barrier surrounding blood vessels by secreting matrix metalloproteases (MMP) to cleave key elements of the basal lamina (63) and undergo cytoskeleton remodeling to transmigrate between the endothelial cells as they move into the vessel (64). These characteristics required for active intravasation are also necessary for successful mobile foraging. A cell's ability to manipulate its environment and itself to move in search for resources predisposes it to overcoming intravasation barriers. In this way, the entry barriers for blood and lymph vessels select for mobile foragers while keeping cells that lack the required characteristics out of the dispersal corridor thus preventing them from dispersing.

The third dispersal corridor, the nerve, is analogous to a railroad track with clear physical delineations, but lack of directionality. Prostate cancer cell entry into this corridor is called PNI, which is the invasion of prostate cancer cells in, around, and through the layers of the nerve (29). Nerves lack a surrounding matrix or cell layer and thus do not have high barriers to entry, but do require

cells to move as mobile foragers to enter and traverse the nerve corridor. Once again a high movement foraging strategy proves to be a prerequisite for entry and long-distance dispersal.

Selection pressures of long-distance dispersal within corridors

Although all cells can enter corridors via passive sloughing, not all can survive the corridor barriers to dispersal. Corridors, such as rivers and railroads, are primarily suitable for dispersal as opposed to colonization as their conditions may provide just enough resources to allow the dispersing organism to stay in the corridor but are not suitable habitats for colonization. Likewise, blood vessels, lymph vessels, and nerves permit survival but not colonization. Only individuals with certain characteristics can disperse via these corridors and these individuals are selected for by the conditions of each corridor.

Long-distance dispersal along a corridor characterized by a unidirectional current, such as a river, selects for individuals with the ability to survive the drastically unfamiliar current conditions. In a prostate cancer primary tumor, cells that have entered the lymphovascularity must withstand increased predation risk and current forces during dispersal. Dispersing cells therefore are selected for a number of survival characteristics: immune evasion, ability to withstand sheering forces (64, 65), and resistance to anoikis (induction of apoptosis due to a cell's detachment from the extracellular matrix; refs. 56, 66). These barriers to dispersal act as selective pressures to limit successful dispersal to cells with the required characteristics.

In the case of unidirectional current corridors, the ability of the cell to move independently is not required because the current acts as an extrinsic displacement force. In contrast, in immobile delimited corridors, such as the railroad tracks used by coyotes (67), the organism must actively move to utilize the corridor. The nerves in the primary tumor act as stationary corridors: they provide a path along which the cell can travel but do not provide an external force to facilitate movement. To successfully disperse along this type of corridor, an individual must be able to transport itself along the corridor path. Thus, nerve dispersal selects for mobile cells that can survive the nerve fiber environment and locomote along the corridor. Thus, the adaptive mobile foraging phenotype is selected for in an ideal metastatic "seed" and is evidenced by the positive association of PNI and development of bone metastasis (28).

While some tumor cells use the nerve and lymph as corridors for dispersal from the primary tumor, eventually all dispersing cancer cells enter the blood circulation (Fig. 4). Cancer cells that leave the primary tumor via nerve corridors likely use the nerve-surrounding lymphatic space in the prostate to enter the lymph circulation, joining the cells that originally left the primary tumor through the lymph corridor (26, 68). Transferring to the lymph corridor induces lymph-associated barriers that were not present in nerve dispersal. Thus, to successfully disseminate, even cells that escape the tumor via nerve must also possess the characteristics required for entry and survival in a one-dimensional corridor (i.e., immune evasion, resistance to anoikis, etc.). Cells dispersing in the lymph pass through lymph nodes before draining into the blood. Some dispersing prostate cancer cells in the lymph will be trapped in and colonize the lymph node (28, 69), resulting in regional lymph node metastasis.

Jump dispersal of prostate cancer cells through the vasculature

Eventually, all dispersing prostate cancer cells enter the venous blood circulation (Fig. 4). Once in circulation, circulating tumor

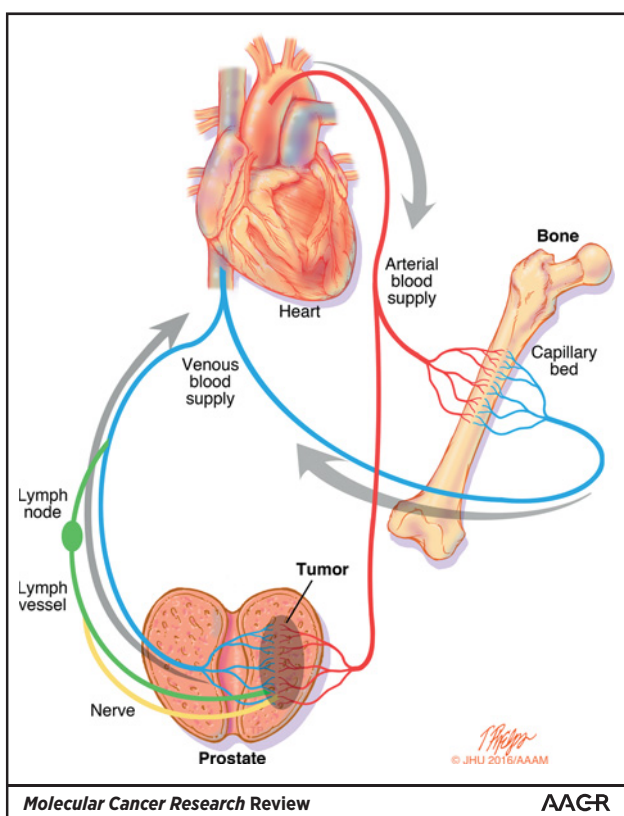


Figure 4.

Path of dispersing prostate cancer cells from the primary tumor. Prostate cancer cells disseminate from the primary tumor via venous blood vessels (blue), lymph vessels (green), or nerves (orange). Nerve-disseminated cells enter the lymph and all cancer cells in the lymph pass through at least one lymph node before entering the venous blood supply. CTCs are then carried through the body via the blood circulation. CTCs pass through the heart and lungs to enter the arterial blood supply. CTCs are carried with the blood through the arterial system, entering distant organ capillary beds at random. Upon reaching a suitable secondary site, such as the bone, cells must extravasate from the blood vessel to colonize the metastatic site. Image printed here with permission from the source: Tim Phelps ©JHU/AAAM 2016, Department of Art as Applied to Medicine, The Johns Hopkins University School of Medicine.

cells (CTC) are outwardly indistinguishable from each other regardless of initial dispersal corridor or mode of entry into the corridor (passive or active; refs. 69, 70). After entering the blood circulation, cells undergo a high velocity dispersal resembling the ecological phenomenon of jump dispersal, the rapid, long-distance dispersal of an organism across inhospitable habitat (71). In ecology, successful jump dispersal events are rare but are required for dispersal to distant habitats. For example, a jump dispersal event was required for the spread of monkeys from Africa across the Atlantic Ocean to South America (72). Similarly, jump dispersal through the blood is a critical event for the establishment of distant prostate cancer metastases corresponding to CTCs and bone metastases in patients (73).

Jump dispersal provides additional selective pressure to corridor dissemination. As cells enter the larger blood circulation, current velocity increases and, as a result, shearing forces increase (74, 75). Thus, successful jump dispersers must

withstand much greater current velocities and shearing forces. Only cells with the characteristics required for overcoming these jump dispersal barriers will be able to successfully disseminate to distant organs such as the bone. Thus, jump dispersal events in prostate cancer metastasis select for cells that can enter the blood circulation and survive its inhospitable conditions.

Dissemination destination is stochastic and requires permissible "soil" for metastasis

To understand the interconnectivity of the blood, lymph, and nerves, and the long-distance dispersal of CTCs, it is important to view the circulation as a one-way circuit with all the corridors merging into the systemic circulation (Fig. 4). In order for a clinical bone metastasis to arise, a dispersing cancer cell from the primary tumor in the prostate must travel through one of the dispersal corridors and eventually undergo a jump dispersal event into the venous circulation. Venous blood carrying the CTC travels through the heart and lungs and exits the heart as part of the arterial blood supply. The CTC is carried by the blood flow to distant regions of the body: potential secondary sites of metastasis. Importantly, the dispersing CTC does not have explicit control over its direction or final destination and "homing" to a specific site while in the circulation as a CTC is impossible (32). Therefore, landing in a favorable habitat is a stochastic event. A CTC is just as likely to disseminate to a bone capillary as a liver or muscle capillary.

Wherever the CTC lands, to survive, the cell must extravasate from the circulatory vessel and survive in the secondary site environment as a disseminated tumor cell (DTC). For a clinical metastasis to arise, the DTCs must proliferate and colonize the secondary site, often observed years or decades after the primary tumor was removed. The success of this metastatic event, detailed by Stephen Paget's "seed and soil hypothesis" is largely dependent on the characteristics of the secondary site "soil" that must permit or promote colonization. As prostate cancer preferentially develops clinical metastasis to bone (58), it is likely that the bone "soil" is highly favorable for prostate cancer DTC colonization or for DTC reawakening to establish a clinical metastasis.

Prostate cancer metastasis is an inefficient process

The journey from foraging in a local patch in the primary tumor to colonization of a secondary site is laden with selection pressures making the metastatic process incredibly inefficient (Fig. 5). A successful metastatic event requires a cancer cell to survive in the primary tumor habitat, encounter a dispersal corridor, enter the corridor, disperse along the corridor, jump disperse through the circulation, land in a permissive secondary site, extravasate, and colonize (Fig. 1).

On the basis of the current detection techniques, men with metastatic prostate cancer have as many as 5,000 cells in circulation at any given time (76). Assuming that a CTC will only survive a single pass through the circulation (as evidenced by the relatively low CTC count per total tumor burden) a prostate cancer tumor produces approximately 7 million CTCs per day. Despite the high numbers of CTCs introduced into the dispersal corridors, only a rare number of those CTCs are successful bone marrow DTCs, and even fewer are the seed for a clinical metastasis. For a single DTC to arise 10 years after primary tumor formation, more than 15 million CTCs would have been released from the primary

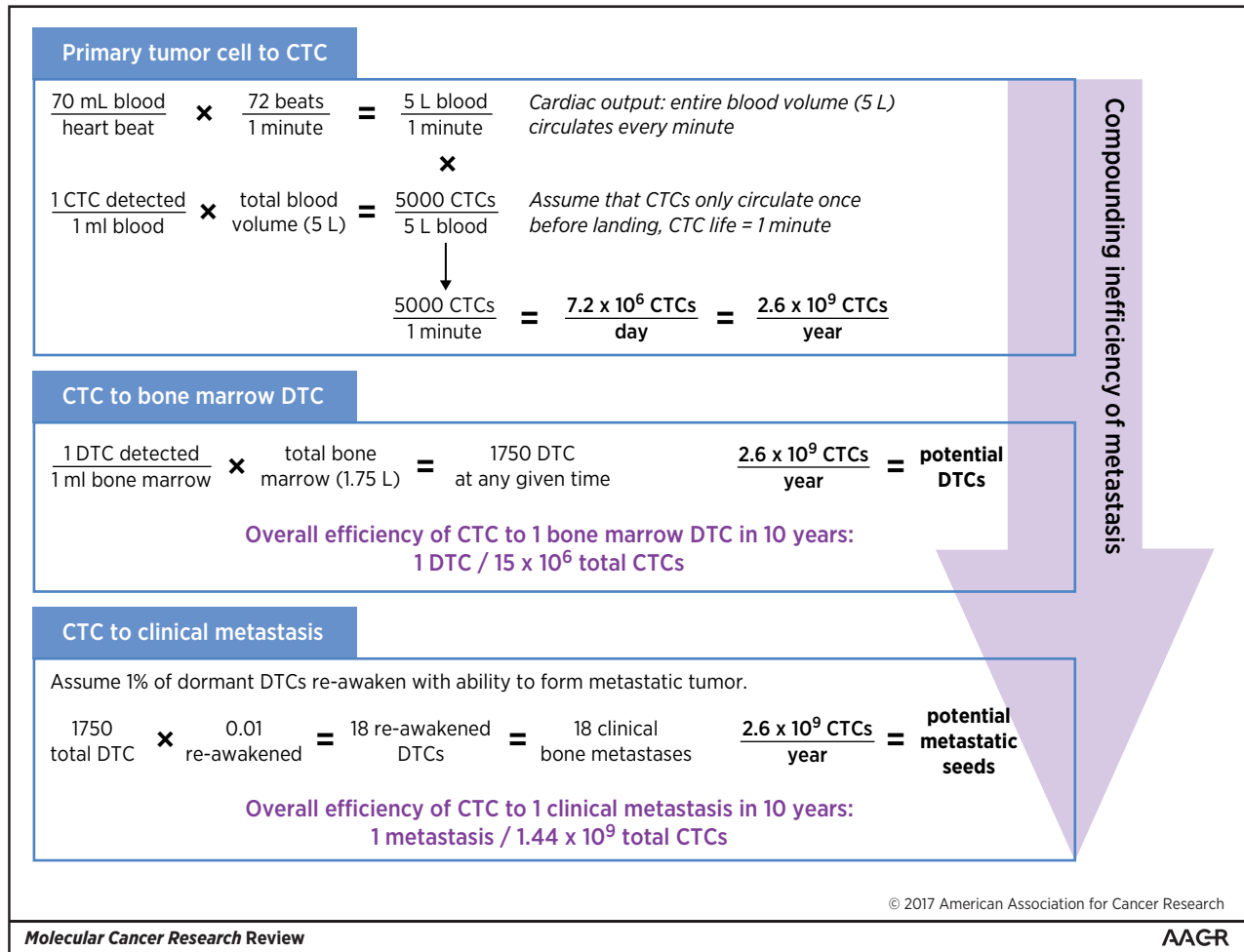


Figure 5. Inefficiency of prostate cancer metastasis. Of the cells that disseminate from the primary tumor (detected as CTCs from the venous circulation), very few become bone DTCs and even fewer eventually form clinical metastases. The inefficiency of each of step compounds with progression along the metastatic process.

tumor. Even more striking, the likelihood of a CTC seeding a metastasis over 10 years is 1 in 1.44 billion (Fig. 5).

The incredible inefficiency of metastasis underscores the necessity of a specialized subset of adaptations in order for a primary prostate cancer cell to successfully metastasize. Such adaptations arise from the selective pressures faced with each step in the metastatic cascade, including within the primary tumor and during dispersal. This highlights the necessity of understanding the unique environmental pressures of the selective "soil" to give rise to metastatic "seeds."

Metastatic "seeds" are likely mobile foragers

The success of a metastatic "seed" is dependent on the cell's ability to escape the primary tumor and colonize an unfamiliar secondary site. While some prostate cancer cells, including stationary foragers, exit the primary tumor through passive sloughing, it is unlikely that these cells will exhibit the necessary adaptive phenotype to equip them to survive dispersal or to thrive within a metastatic site. In contrast, mobile foragers, such as those with the capability to disseminate along the

nerve, possess the adaptations required for overcoming selection pressures encountered along the metastatic cascade and therefore are selected for as metastatic "seeds." The adaptations accumulated by mobile foraging prostate cancer cells in response to the selective pressure of the primary tumor primes the cancer cells to (i) encounter a greater number of dispersal corridors, (ii) survive the severe dispersal event, and (iii) have the capacity to invade a secondary site.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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