Is cancer a disease of self-seeding?

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The high cell density, rapid growth rate and large population size of cancer are conventionally attributed to a pathologically high ratio of cell production to cell death. Yet these features might also or instead result from inappropriate cell movement, already understood to underlie invasion and metastasis. This integrating concept could induce a broadening of our existing anticancer pharmacopoeia, which, with mitosis as its predominant target, is now seldom curative.

Although epithelial cancers are diverse genotypically and phenotypically, several features are universal[1–5].One of these is the abnormal capacity of cancer cells to migrate, which manifests in two ways. The first is invasion: the cells' ability to become dislodged and travel within the tissue of origin. The second, metastasis, involves travel beyond the tissue of origin. Aberrant cell mobility is thought to be distinct from (while sharing some molecular pathways with) another universal feature: an increased ratio of proliferation to cell death[1,6,7]. This latter paired abnormality, rather than cell mobility, is thought to underlie tumorigenesis, the expanded generation of cancer cells that produces large, destructive masses. So, the current view of cancer incorporates a set of overlapping dichotomies: invasion versus metastatic spread, both relating to cell mobility; tumorigenesis versus metastatic behavior, the former thought to result from cell proliferation, the latter from cell movement.

Here we seek to unify these dichotomies by considering the possibility that pathologic cell mobility, in addition to being crucial to tumor invasion and metastatic dissemination, can also contribute significantly to primary tumor growth. Moreover, the mechanism by which cell mobility can increase tumor size can also produce high cell density and rapid growth rate.

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We call this proposed mechanism 'self-seeding', a term used in botany to describe the ability of plants to overgrow an ecosystem. Consider how a mass of weeds dominates a field: not by the massive increase in size of individual weed plants, but rather by the continuous propagation of new weeds both within the weed bed (density) and at its periphery (invasion). In cancer, this concept would translate to escapee cells that constantly re-seed a tumor mass, making that mass a dense collection of contiguous small growths that infiltrate and potentially destroy its host organ. Furthermore, just as a self-seeding weed population might spread beyond its field of origin, so might cancers seed distant metastases.

The logic of self-seeding

Many of the faculties that permit distant seeding could logically enable self-seeding (**Fig. 1**). These include separating from an anatomic mooring, lysing a proteinaceous and carbohydrate matrix, intravasating, circulating, adhering to an endothelium, extravasating, attaching in a target location, inducing angiogenesis and propagating in the target environment[1]. Seeds might depart from the primary site of tumor formation or from a metastatic site. Returning to the site of origin would constitute self-seeding or re-seeding, whereas lodging in another (distant) site would constitute the process of metastasis.

Cancer invasion is symbolized in **Figure 1** by pathways A and D. Metastasis is symbolized by pathway C. Here we hypothesize the existence of pathways B and E, through which a dislodged, wandering cancer cell, having access to the entire systemic circulation, has a finite probability of returning to its site of birth. To be sure, this return trip could well be faced with an unfavorable circulation pattern, including encounters with intricate, tortuous capillary beds that may retain the wandering cells before they can return to their site of origin. Those cells that do manage the return trip, however, would find themselves in the welcoming microenvironment in which they first developed or in which they took root[1–5]. The co-evolution of cancer cells and their stroma that creates such a microenvironment is often termed 'field cancerization', and may be reflected in the presence of tumor and stroma-specific gene expression patterns[8–12].

The molecular mediators of self-seeding in the primary site (pathways A and/or B) and self-seeding in metastatic sites (pathways D and/or E) might be partially overlapping. Successful self-seeding might well require the continual generation of self-renewing progeny. Therefore, we envision that some proportion of self-seeds may be acting the role of, or be synonymous with, cancer 'stem' cells, more accurately called tumor-initiating cells[13,14]. And, to the extent that normal stem cells may undergo tumorigenic mutations, their intrinsic self-renewing and migratory properties might turn such cells into highly effective self-seeds.

An appealing aspect of the concept of self-seeding is that it could explain diverse characteristics of cancer, extending the analogy to a weed-strewn garden. Dysplasia could be the consequence of the tumor being a spatially disoriented conglomerate of functionally independent smaller masses. As self-seeded loci continue to self-seed, the result would be more and younger loci of new growth and hence more histologic disorganization as well as a preponderance of immature cells. Furthermore, independent masses at the periphery of a cancer would be *de facto* points

of invasion whether they arose by pathways A or B in the primary site, or pathways D or E in a metastatic site. Each new growing component of the conglomerate would tend to attract its own independent blood supply, promoting hypervascularity[15]. If self-seeds return to the primary tumor's organ of origin but do not attach to the mass of the primary tumor, this would convey the appearance of multifocality. (Of course, true multifocality—multiple points of primary carcinogenesis—may also coexist with this process.) As the cells establishing these noncontiguous seeded loci are samples of the heterogeneous collection of cells already present, they may be sufficiently distinct that they convey the appearance of polyclonality. Moreover, once established, satellite lesions could further evolve and intermingle, consistent with the observation that multifocal lesions often seem to be polyclonal. Self-seeding might also be relevant to the hotly debated question of whether a primary tumor exhibits the expression signature of its metastatic descendents: self-seeding would tend to equalize the molecular profiles of an aggressive tumor and its metastases, their degree of similarity increasing proportional to the degree to which self-seeding accounts for the growing mass of the primary tumor.

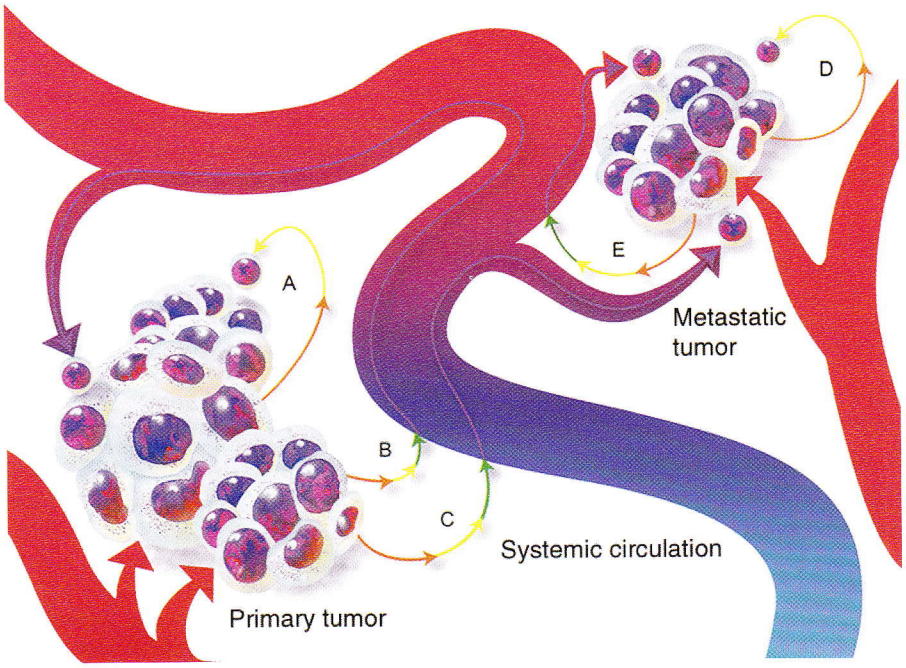

Figure 1 The self-seeding concept of cancer growth and metastasis. The primary tumor mass on the lower left is a composite of three smaller sub-tumors: the central, larger one is a proliferating collection of cells at the original site of carcinogenesis; the other two sub-tumors on the lower left are the progeny of self-seeds that spread from that original site. Because each of the three is relatively small compared with the volume of their sum, they are relatively both dense and rapidly growing (see text). A self-seed to the primary tumor may follow pathway A, comprised of dislodging (orange arrow) and proteolysis of the extracellular matrix (yellow arrow) followed by reattachment in or at the primary site, then proliferation and angiogenesis. Or the self-seed might follow pathway B, which includes intravasation (green arrow), circulation and extravasation (purple arrow), then return to the primary tumor bed, proliferation and angiogenesis. A cell following pathway C traces the same steps, but relocates to a metastatic site, where growth can occur by proliferation, angiogenesis and self-seeding at that site, either directly (pathway D) or via circulatory flow (pathway E). The expressed gene sets responsible for these processes may be shared completely or partially. For example, pathways B and C may require the same biochemical processes, and hence the same gene expression pattern, with the final destination (primary or metastatic sites) determined stochastically. Or pathway C may use the same genes as pathway B, but with some additional properties. The genes responsible for pathways D or E may be significantly overexpressed, whereas the genes associated with pathways A or B may not, which would explain virulent growth in a metastatic but not the primary site. Many other patterns of local and distant growth are consistent with the model, as discussed in the text.

The mathematics of self-seeding

Self-seeding could explain increased cell density, high mitotic rate and large tumor size by reference to two related biomathematical concepts: fractal geometry and Gompertzian kinetics. Biological structures are best described mathematically by fractal geometry rather than the Euclidian geometry of regular solid masses like spheres and cubes[16,17]. By Euclidian geometry the volume of a biologic mass increases by the cube of its diameter. In contrast, by fractal geometry the number of cells in that mass increases by a power constant less than three. Hence, the ratio of cell number to volume (termed 'cell density') falls as the volume increases. Because of this, a fully developed normal organ, or even a large section of that adult organ, would in most cases have a lower total cell density than the whole organ in its primordial, smaller state. In contrast, if—as self-seeding would suggest—a cancerous mass arising from that organ is a conglomerate of small, incipient, component masses, each would have a small volume and hence a high cell density, creating a high cell density in the cumulative whole. (An important exception to this general rule is discussed below.)

The abnormally high cell density found in most cancers is labeled 'hyperproliferation' because its microscopic appearance—more cells per unit of space—is regarded properly as evidence of more cell divisions in conjunction with relatively fewer cell deaths. This does not, however, imply that the regulation of mitosis or apoptosis is necessarily abnormal. An alternative cause of high cell density could be the cells' normal response to the 'start-up' nature of the small tumor seedlings comprising the malignant conglomerate.

One of the consequences of high cell density is a high density of proliferating cells. This might be one reason why small-volume masses grow more rapidly relative to their sizes than masses of larger volume, as first described mathematically by Benjamin Gompertz[18]. This phenomenon results in a sigmoid growth curve on an arithmetic plot of size versus time, with population size eventually approaching a plateau phase of slow to imperceptible further growth (**Fig. 2**). Gompertzian kinetics has proven useful as well as applicable in the clinic, particularly in the design of improved cancer treatment regimens[18].

If a malignant tumor is a conglomerate of component Gompertzian masses, each with a relatively high growth rate because it is relatively small, then the whole conglomerate—being the sum of its parts—would have a high growth rate as well. In addition, a conglomerate of small Gompertzian tumors would grow large because it would sum the many individual Gompertzian plateaus of the components. Hence, the concept of self-seeding provides an explanation for both the increased growth rate and the increased total volume of malignancy without needing to hypothesize deviant regulation of mitosis or cell death at the individual cellular level.

The relationship between proliferation and self-seeding

The above analysis does not ignore the obvious facts that abnormalities in mitotic regulation and an aberrant ability to survive such stresses as cell-cycle checkpoint alterations, hypoxia, glucose deprivation or interstitial fluid pressure changes are commonly found in cancer cells[1,6]. We merely indicate that abnormal cell mobility could contribute to phenomena previously attributed entirely to other processes. Furthermore, it is possible that hyperactive mitogenic pathways and evasion of growth inhibitory constraints may be necessary but not sufficient for overt carcinogenesis. Hyperproliferation is also common in many benign conditions such as dermatoses and premalignant lesions. Furthermore, mice genetically engineered to overexpress purely mitogenic oncogenes characteristically demonstrate benign hyperplasias in many organs[7,19]. True cancers arise from some, but only some, of the cells in these genetically altered organs. To become malignant, therefore, tumors require additional abilities, which may be present from the outset (for example, in normal tissue stem cells that undergo transformation) or may be acquired later. We argue that one of these abilities is the capacity to self-seed. In this regard, the observation that some potent oncogenes simultaneously disrupt both cell adhesion (part of the seeding process) and mitotic-apoptotic regulation may explain their efficiency in causing virulent cancer[7,19]. That the expression of matrix metalloproteases (also part of the seeding process) may be sufficient to cause malignant transformation in transgenic mice is similarly supportive of our concept[2–5].

Increased proliferation is clearly an adverse prognostic marker in many types of epithelial cancer[20]. Although this is usually thought to reflect the cancer cells' mitotic-apoptotic dysfunction, we may wish to consider that it also could reflect the aggressiveness of self-seeding. As more vigorous self-seeding would produce more loci of growth within the tumor conglomerate, it would result in faster overall growth rate as well as larger potential total tumor volume. Furthermore, and perhaps most importantly, a self-seeding etiology of hyperproliferation would correlate well with a tumor's metastatic ability, which is, after all, the prime cause of poor prognosis in clinical cancer.

This line of reasoning may cast new light on one of the clinical truisms of epithelial cancer, that large primary tumor size is a poor prognostic feature. The conventional view is that cancers gain metastatic ability through an accumulation of mutations as they grow to large size. Yet perhaps some tumors grow to be large, and have a poor prognosis, because they seed aggressively to self and to distant sites. Cancers may not be bad because they are big; they may be big because they are bad.

The molecular links between metastasis and self-seeding

Lending credence to the mathematical reasoning above is recent molecular evidence that the ability of a wandering cancer cell to re-seed its original mass may be linked to the ability to seed distant sites. Primary tumors frequently express genes whose products alter the microenvironment, such as extracellular matrix proteases, glycosylases, proangiogenesis factors, regulators of cell adhesion, and mediators of inflammation and angiogenesis[1–5,7,9–14,19]. Gene expression signatures that include these genes predict metastatic behavior and hence poor prognosis in breast cancers[9–12]. Indeed, so important is the ability of a cancer to alter its environment that poor-prognosis gene-expression signatures are emerging which largely exclude genes that purely mediate proliferation or survival[11,12].

It is commonly assumed that these environment-altering genes are associated with poor prognosis only because they are responsible for metastatic behavior. However, although human breast cancer xenografts with high metastatic capacity in the laboratory do demonstrate a gene expression profile associated with metastatic behavior (and hence poor prognosis) in the clinic, so do cell clones with less prominent metastatic behavior[19,21,22]. We therefore consider that the genes in the 'poor-prognosis' environment-altering set may underlie the self-seeding that permits growth in the primary site and also lay the foundation for distant seeding. It should be expected, therefore, that genes in addition to those in the poor-prognosis set are needed to promote growth in metastatic sites, and in fact there is evidence that this is the case. In highly metastatic laboratory models of human cancer, there are genes that are both highly expressed and responsible for site-specific metastases[22]. Some of these genes are also found to be expressed in human primary tumors that show a high risk of developing metastasis to the lung[22].

Of particular importance in this regard is the experimental evidence that a subset of lung metastasis–associated genes also contribute to tumor growth in the mammary gland[22]. This finding may be interpreted as follows: these particular genes may act not only as metastasis-producing, distant-seeding genes (pathways C and possibly D and/or E in **Fig. 1**), but also as tumor self-seeding genes (pathways B and possibly A). Furthermore, a different and distinct gene subset is associated with the growth of metastases in the lung but not in the primary site[22]. Perhaps some of these genes, which might be associated with pathway C, are causing self-seeding (pathways D and/or E) in the metastatic masses but not in the primary site (pathways A and/or B).

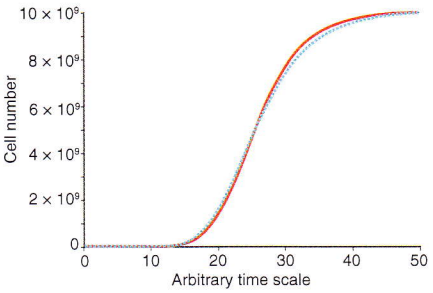

Figure 2 Gompertzian growth curves. This pattern of growth is ubiquitous in nature. As the number of cells increases with time the growth rate relative to the number of cells decreases, eventually approaching zero as the number of cells approaches a limiting plateau size (10^{10} in this example). If a tumor is comprised of many Gompertzian masses, each arising from a self-seed, rather than one mass, as would occur in the absence of self-seeding, the conglomerate's maximum size will be the sum of many plateaus, which could be very large, possible even lethal. Self seeding might cause Gompertzian growth itself. Gompertz's original equation is shown as the solid line[18]. The dashed line, which is almost indistinguishable from the solid line, is a new equation (below) in which proliferation occurs in an anatomic region of the mass that has a lower fractal dimension than the region where apoptosis occurs. Self-seeding would create such a pattern since the seeds, approaching the mass from the outside (pathway B in **Fig. 1**) or migrating outward (pathway A), would congregate on the periphery (or leading edge) of the growing mass rather than the mass's volume. $dN_t/dt = h_1 \cdot N_t^{(a/c)} - h_2 \cdot N_t^{(b/c)}$. The constants are $h_1 > h_2 > 0$, and $3 \geq c \geq b > a \geq 2$. (In this example, a = 2.8, b = c = 2.9.)

Some natural history implications of self-seeding

The concept that many genes may be involved in these processes could explain some of the complex and enigmatic phenotypes that are observed in nature. For example, one could envision a cancer that expresses metastasis-permissive genes (pathway C in **Fig. 1**) but lacks prominent capability to colonize metastatic sites (pathways D and/or E). This would result in the production of latent metastases restricted in their volume expansion. Perhaps this is why some individuals with breast cancer who have cells of epithelial origin in their bone marrow, presumed to be breast cancer metastases, never demonstrate clinically significant osseous disease.

Another eccentric phenotype would be that of a cancer with such intense self-seeding

power (pathways A and B) that it would never form a discrete tumor mass in the primary site, but rather demonstrate a diffuse infiltration of the organ of origin. This would provide an exception to the general rule cited above that cancer cells form dense masses. As the genes that underlie such behavior may well be associated also with pathways C, D and E, we should expect to see virulent metastases in many distant organs. Many adenocarcinomas of the pancreas may act in this way. In other cancer types, metastases in organs with especially cancer-supporting stroma may also exhibit such behavior. If a cancer's pathways C and D and/or E are most prominent, the disease could become widely metastatic while never demonstrating a large or even a discernable primary mass. Indeed, on rare occasions we do observe breast and lung cancers that act like this. Another possibility is that a primary cancer could spin off a cell type with the capacity to strongly self-seed (pathways D and/or E) only in one spot in one distant organ. In such a case the individual would benefit considerably from resection or irradiation of that solitary metastasis in addition to control of the primary tumor. Hence, variations on the themes depicted in **Figure 1** could well encompass diverse clinical presentations.

It must be emphasized, however, that all of the above examples of discrepancies in growth characteristics between primary and metastatic sites are exceptions to the general rule. In the vast preponderance of cases of clinical cancer there is such a consistent association of large tumor size, anaplasia, rapid growth rate and metastatic behavior that it leads one to hypothesize, as we have, that the molecular roots of these phenomena are likely to be the same or very closely related.

A therapeutic implication of self-seeding

Because of our community's historic focus on cell proliferation as the core aberrancy in cancer, almost all anticancer drugs now in common use were developed as antiproliferative interventions. These drugs have unquestionably proven useful because they shrink tumors, which improves prognosis by postponing death or delaying recurrence after resection[18,20]. Cure of established epithelial cancers is uncommon, however, and even the advances we have made in the use of postsurgical adjuvant drug therapy may reflect time delays (as regrowth proceeds from residual cells) rather than eradication of all cells in some individuals with cancer[18]. Moreover, whatever benefits are gained from the use of antimitotic drugs are often at the cost of considerable toxicity because cellular proliferation is so intrinsic to the viability and function of most normal tissues.

The fact that our current therapeutic strategies have been largely disappointing to date should not surprise us if further research confirms that seeding as well as mitosis is at the core of malignancy. Only recently have clinical oncologists started to explore agents directed against targets other than those involved in cell proliferation. Antiangiogenesis is one such example. In these clinical trials, however, our bias toward mitosis as a primary target is evident in our tendency to combine new therapeutics with antiproliferative agents, usually chemotherapy[23–25]. This may not be an ideal long-term strategy. In addition, neoangiogenesis is only one of many possible targets along the paths to successful self-seeding, and may not be the best, as it is far down the pathway. But as we develop antiseeding drugs we must beware of new toxicities, as we might be disrupting certain crucial physiological processes—wound healing and gamete production being examples.

Conclusion

Experimental and theoretical results are suggesting the possibility that the process of self-seeding, a putative close relative of the process of metastasis, may influence or underlie many important features of epithelial cancer. These include high cell density, invasion, multifocality, amplified growth rate, enlarged cell-population size, and the capacity to spread to and reproduce these features in distant organ sites. Testing the validity of this hypothesis will require a combination of approaches. For example, it would be of interest to see whether tumor size is causally linked to the effect of metastasis-gene expression signatures. We also need to determine whether metastatic tumor masses have the ability to attract circulating cells released from the same tumor, and whether this self-seeding ability, contributing to tumor growth, depends on pro-metastatic genes. Yet if validated by these or other means, this new concept would help us to better understand malignant diseases and perhaps to discover how to manage them more effectively.

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