Clinical implications of cancer self-seeding

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Abstract | Most metastatic cancers are incurable—a fact that underscores the limitations of our existing paradigms for understanding metastasis. In this Review, we use breast cancer to explore many of the enigmas revealed by these existing paradigms. Traditionally, metastatic models describe metastasis as a unidirectional process, whereby cancer cells leave a primary tumor and unidirectionally seed metastasis in regional lymph nodes or distant sites. By contrast, recent data indicate that metastasis is a multidirectional process whereby cancer cells can seed distant sites as well as the primary tumor itself. This later process, known as 'self-seeding,' has been validated in diverse experimental models. Here, we show that the self-seeding model may answer many of the mysteries inherent to cancer metastasis. Indeed, reframing our understanding of metastasis within the self-seeding model offers new opportunities for prevention and cure of metastatic cancer.

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Introduction

To function on a day-to-day basis, cancer clinicians have learned to accept many enigmas. These are defined as sets of observations that are appreciated to coexist despite being incompatible with each other under prevailing theory. The history of science has taught us that such enigmas are often relieved by new observations that generate new theories to replace the old theories. We are now at such a juncture, motivated by new observations concerning the metastatic behavior of epithelial—and perhaps other—cancers.

In this Review, we use examples from breast cancer to consider several enigmas in cancer medicine, and examine how these enigmas might be reconciled by recent findings in the laboratory. The hypothesis generated by these new observations has been termed self-seeding, referring to the proven ability of peripatetic cancer cells to seed not only to regional and distant sites in the body, but also the original source: the tumor itself.[1,2] The hypothesis is based on biological, clinical, and bio-mathematical considerations—but is primarily rooted in studies of cancer cell biology and chemistry. While this is still a young hypothesis, it seems possible that in addition to solving several mysteries in clinical cancer, it might have constructive applications in both diagnostics and therapeutics.

Enigmas in cancer medicine

Similarities between pre-cancer and cancer

One mystery is the gross molecular similarity of preneoplasia and invasive cancer, which contrasts sharply with their divergent natural histories. For example, ductal carcinoma *in situ* (DCIS) lesions of the breast do not metastasize and seldom form large masses, whereas invasive ductal cancers, if not treated successfully, kill by metastasizing and destroying resident organs due to unrestrained growth. We know that invasive cancer cells manifest a myriad of changes including gene mutations, rearrangements, amplifications, deletions, and RNA-expression patterns.[3] This genomic diversity is thought to underlie phenotypic diversity in self-sufficiency in growth signals, insensitivity to antigrowth and pro-apoptotic signals, and the ability of tumors to invade, metastasize, elicit neoangiogenesis, and to replicate limitlessly.[4] Yet, DCIS cells demonstrate comparable molecular changes, indicating that major biological differences must be rooted in minor chemical changes.[5]

Mammographic breast density: a risk factor

It is well known that the highest risks for the development of breast cancer are found in women with germline mutations in the DNA-repair genes *BRCA1* and *BRCA2*.[6] Second only to these risk factors is mammographic breast density.[7] Breast density is largely a measure of inert stromal tissue, especially collagen, which is thought to be merely a supportive structural element.[8] Postmenopausal lobular involution of the breast has been shown to lower the risk for breast cancer,[9] but this lowered risk correlates weakly with mammographic breast density.[10,11] Hence, the mechanism by which a structural component that is independent of mitotic rates or other 'logical' mechanisms of carcinogenesis influences breast cancer risk remains an enigma.

Coexistence of phenotypic properties

On the microscopic scale we find another mystery: traits always found together despite their diverse genetic etiologies. Tumor size, histologic grade, and propensity to metastasize (and hence kill) are strongly linked.[4] Why should genetic abnormalities that increase growth rate always be found with different genetic abnormalities that control microanatomic architecture and other abnormalities that underlie metastases?

Competing interests
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Key points

- Most metastatic cancers are currently incurable; thus, existing paradigms for understanding metastasis reveal persistent enigmas
- In breast cancer, unsolved mysteries include the similarities between pre-cancer and cancer, mammographic breast density as a risk factor, and the relationship between local control and distant recurrence
- A new paradigm, termed 'self-seeding,' reconciles many of these enigmas
- Self-seeding describes the multidirectional capacity of cancer cells to seed distant organs as well as self-seed a primary tumor
- Reframing drug development and clinical investigation within a self-seeding model may be a harbinger for clinical progress in curing metastatic cancers

Paradoxical patterns of metastatic spread

On the macroanatomic level, how can metastatic pathways be predictable and unpredictable at the same time? Toward the end of the nineteenth century, William Halsted articulated the idea of the predictability of metastatic spread. His concept forms the basis for breast cancer surgery to this day. He accepted the then prevailing concept that cancer cells originating from the breast needed to pass through the lymphatic system to gain access to the systemic circulation. Accordingly, he asserted that malignant spread could be prevented by the meticulous removal of a whole breast surrounding the tumor as well as its attached ipsilateral axillary contents.[12] The validation of the concept seemed to come from the undisputed fact that these so-called radical mastectomies did indeed cure some individuals, whereas patients not treated with radical surgery almost invariably died of their disease.[13] This concept was also validated by the finding that lymphatic invasion is a powerful negative prognostic variable.[14]

Halsted's notion would have remained unquestioned were it not for the work of Daniel Martin Shapiro, Bernard Fisher, Edwin Fisher and colleagues who built a compelling alternative theory from the observation that patients without axillary lymph-node involvement could still develop distant metastases.[15,16] They hypothesized that hematogenous as well as lymphatic pathways for metastases were operant, and hence only systemic therapies—such as antihormonal drugs or cytotoxic chemotherapies—could improve prognoses, not further radical local surgeries, as proposed by others.[13] This idea spawned major interest in peri-surgical drug adjuvant studies, the success of which would have seemed to confirm the alternative view were it not for a later development that has also altered our approach to breast cancer management: sentinel lymph-node mapping.[17,18] This work demonstrated that normal lymph and cancer cells exiting the breast have a common flow pattern. Moreover, if the axillary nodes first exposed to that efflux do not contain cancer cells, the odds are close to certainty that the rest of the axillary contents will be clear, and the attendant prognosis for the patient would be excellent.[17,19] Hellman recognized the vast heterogeneity of breast cancer, whereby local growth, metastases to regional lymph nodes, and metastases to distant sites were correlated but this correlation was imperfect.[20] Hence, Halsted's idea of an anatomic pathway for metastatic spread is correct, and so is Fisher's idea that spread does not require this pathway. How may these opposing views be reconciled?

Linking local control and distant recurrence

There is another enigmatic aspect to the relationship between local and systemic disease. Breast cancers can be widely disseminated as solitary cells or micrometastatic colonies by the time of their initial presentation, as demonstrated by the success of systemic adjuvant drug treatments in improving prognoses. So, local control should not be the *sine qua non* for systemic control: yet it is! Extensive experience—thoroughly analyzed—has unequivocally shown that better local control, such as radiation therapy for treating residual breast tissue following breast-conserving cancer surgery, reduces the odds of systemic as well as local recurrence.[21] The effect is not minor: for every four cases of local recurrence that are prevented by radiation therapy one patient is protected from distant, eventually fatal metastases.[21]

This paradox raises the question of why radiation therapy is needed for local control following the excision of a breast cancer with clear margins, if all known disease has already been removed surgically. In fact, detailed microscopic analysis has revealed breast cancer cells many centimeters away from clear margins; but how did these cells reach these locations, and what is the relationship between these cells in the breast and the eventual appearance of distant metastases?[22]

Further data reveal additional unexplained findings. Data collected over many decades have established beyond question that axillary lymph-node metastases portend a poor prognosis in primary breast cancer.[23] Recently, it has been shown that should a patient undergoing breast-conserving surgery and radiotherapy for a small, hormone-responsive breast cancer and two or fewer involved axillary lymph nodes not receive an axillary dissection, she has about a 27% chance of having additional, non-resected lymph-node metastases in her axilla. Yet, the presence of these involved nodes does not increase the risk of axillary recurrence.[24]

Equally perplexing is the fact that although cancer cells are often disseminated at the time of cancer presentation in the breast, patients seldom present initially with stage IV disease unless the growth of the primary cancer is ignored for years, or is exceptionally aggressive.[25]

Molecular profiling or the stem-cell hypothesis

Two recent and significant advances in breast cancer science—molecular prognostication and stem-cell biology—together produce a fresh enigma. Cancers differ in their patterns of gene expression, and these differences correlate with clinically meaningful outcome differences, such as disease-free survival, overall survival, and benefit from chemotherapy.[26,27] This information is gleaned from small samples of tumor, which are considered representative of the whole cancer. Yet we are now also predisposed to the notion that only rare cells in a cancer are capable of unlimited proliferation—the so-called cancer-stem cells or tumor-initiating cells.[28,29] For molecular profiling

to be practical, then, these cells would have to be disbursed throughout the cancer mass. How is this achieved anatomically by the growing cancerous mass?

Etiology of Gompertzian growth

Another enigma is that of cytokinetics and growth patterns. It has been demonstrated experimentally and observed in the clinic that the growth pattern of breast cancer cannot be explained by simple exponential or linear kinetics.[30] Alternatively, therefore, cancerous tumors must follow S-shaped curves intermediate between these two extremes, curves of the type described by Gompertz in 1825.[31,32] For example, mammographic evidence indicates that an average tumor takes roughly 2 years to grow from one cell to 10^9 or 10^{10} cells (equivalent to one to ten cubic centimeters of densely-packed cells). Were this to occur by linear kinetics it would take the tumor another 2 years to double in size, which is unrealistically slow. Were the tumor to have grown by exponential kinetics, it would double in about 3 weeks, which is unrealistically fast. Thus, the growth pattern must be between linear and exponential.[33] Indeed, Gompertzian kinetics have proven helpful in designing improved regimens of chemotherapy for breast cancer,[34–36] malignant lymphoma,[36] and other malignant diseases. However, mitosis does produce two cells from one, so growth very early in a cancer's history must be approximately exponential. This raises the question of how and why as it grows larger a cancer's growth deviates from the exponential pattern.

Growth and metastasis: emerging principles

A rapidly evolving body of work concerning the molecular and cellular biology of growth and metastasis may provide insight into all of these mysteries. The core principles that are emerging are described briefly below.

Cancer cell mobility

With a few key and almost certainly informative exceptions—inflammatory cells in particular—normal cells stay in their proper places in the body. By contrast, one of the cardinal features of cancer cells is their abnormal mobility.[4] Oncogenes often deregulate both cell anchorage and mitosis, providing a molecular link between these two essential processes.[37] Moreover, several sets of gene-expression signatures have been reported to predict poor prognosis in breast cancer and other diseases.[27] Many of the implicated gene products concern alteration of the microenvironment to which normal cells remain adherent. These include matrix proteinases, angiogenesis stimulators, cell-adhesion molecules, and modulators of cell shape and spatial orientation. Perhaps most thought provoking is that gene-expression patterns associated with inflammation—and excluding proliferation genes, which dominate other signatures—are highly prognostic.[38] Hence, there is 'smoking gun' evidence that inflammatory-like cell mobility (leukocytes as well as cancer cells) is a fundamental characteristic of clinical cancer. Importantly, circulating tumor cells are not only known to be present in cancer, but their quantification has been shown to have prognostic significance.[39]

Tumor-initiating cells

Experimental evidence supports the concept that not all of the cancer cells in any tumor are capable of unlimited proliferation.[28,29] This does not mean that a cancer cell without such capacity cannot acquire this property under certain conditions, but at any sampling point, most of the cancer cells in a breast cancer can divide only a limited number of times and hence cannot initiate a new tumor. The cells that are so capable have been termed 'cancer stem-cells', 'tumor-promoting cells', 'tumor-initiating cells', all terms expressing the concept that these cells are roots of the tree that is the cancer. That such cells have defined molecular characteristics at least partially validates the idea that they exist as definable entities. It has been suggested that a fraction of circulating cancer cells—which are quantifiable and such quantification correlates with clinical course—may fit this cellular compartment.[39]

Leading edge versus tumor surface

Many studies have shown that the crucial part of the cancer is its interface with its microenvironment, often referred to, perhaps erroneously, as its 'leading edge' or 'invasive front'. The interaction of the cancer with the surrounding tissue and stromal components is one of the most intriguing aspects of modern cancer biology.[40] It is at the 'leading edge' that cancer cells have transformed from an epithelial (tissue-fixed) to a mesenchymal (mobile) phenotype (called epithelial–mesenchymal transition or EMT), as well as interacting with supportive stromal cells, such as macrophages, endothelial cells, and fibroblasts.[41,42] In addition, angiogenesis is particularly active at the tumor–stroma interface.[43] The possible terminological error is the implication that the cancer cells are growing out from the bulk of the tumor mass into the stroma (hence 'leading') rather than being located at the surface of the tumor by another mechanism. Hence, the term 'tumor surface', in its topological sense, is probably more accurate than 'leading edge.' Examining the tumor surface from a topological perspective challenges existing notions of the tumor border and in turn reframes the concept of the 'inside' versus the 'outside' of a tumor. Since the tumor surface is the microanatomic site of much malignant activity, tissue geometry becomes critical to understanding cancer.

Growth fraction: a weak prognostic indicator

It is an established and well-documented observation that the percentage of cells in a tumor that are demonstrably undergoing mitosis—the so-called growth fraction—is correlated with clinical outcomes, but weakly.[44] Therefore, it is not surprising that highly metastatic and lethal subcultures of human cancer cells do not necessarily have higher growth fractions than cell lines with lower metastatic potential.[2] What was surprising, however, is that in spite of not having a higher percentage of cells in mitosis these cancer cells grow faster in the primary implanted site, the prepared mammary fat pad. This finding is in spite of the fact that gene-expression signatures associated with metastatic behavior do not emphasize aberrations in mitotic or apoptotic regulation, but rather environmental modification.[45–47]

Box 1 | Mathematics of tumor growth

Stem-like cells divide symmetrically or asymmetrically, producing progeny fated to divide M number of times. If a stem-like cell divided asymmetrically, at the same or a slower rate than more mature cells, it would result in a stable size (N_M) between 2^M+1 and $2^{(M+1)}$. For a tumor mass (N_t) to continually grow over time (t) the growth rate would depend on the average cycle times of the cells, but the eventual tumor size (N_∞) would depend only on the eventual number (K_∞) of stem-like cells, since $N_\infty = K_\infty \times N_M$. The growth pattern would depend on how the number of stem-like cells (K_t) changes over time.

For a solid sphere-shaped tumor, where the stem-like cells are on the surface (S), the volume (V) of the tumor would be proportional to the cube of its diameter (L), whereas the surface would be proportional to the square of its diameter ($V \propto L^3$ and $S \propto L^d$, where $d=2$). As the mass increases in size, the surface area to volume ratio (L^2/L^3) drops. The ratio between the new cell production rate and the mass of the bulk of the tumor (K_t/N_t) would also drop as the tumor size increases, leading to a slowing of relative growth.

Biological objects are not true sheets or spheres so the dimensions of their surfaces are between two and three. Hence, $K/N \propto L^d/L^3$, where $2<d<3$. If stem-like cells resided at the tumor surface only, the relative growth rate, proportional to K_t/N_t would slow over time provided that surface had a dimension less than three.

The primary tumor of size N_t is not one mass, but a conglomerate of K_t contiguous masses, each growing to N_M cells on average. The growth rate of CTCs would be proportional to K_t and the growth rate relative to the size of the mass would be proportional to K_t/N_t, which decreases as N_t gets larger. Thus, self-seeding explains the mystery of Gompertzian growth.

Fundamental mathematics of growth

To make sense of this mountain of information it is helpful to discuss certain relevant, evocative yet simple mathematical ideas.[33] The fundamental unit of growth is binary mitosis. Let us consider the prevailing concept that some stem-like cells are capable of unlimited mitoses during the life of the tumor, while others—the progeny of such cells—can divide only a limited number of times before succumbing to cell senescence or death. If an occasional cell not capable of unlimited proliferation were able to convert into a cell with this capacity, this would not alter the fundamental mathematics of tumor growth.[30] The stem-like cells may divide symmetrically—producing two new stem-like cells—or asymmetrically, producing one new stem-like cell and one cell fated to divide say M number of times on average. It is easily shown that were a single stem-like cell to repeatedly divide asymmetrically, at the same or a slower rate than the more mature cells it spawns, it would form a cellular family of stable size (Box 1). Hence, the only way for a tumor mass to continually grow over time would be for the stem-like cells to sometimes divide symmetrically, with a stem-like cell dividing to produce two stem-like cells. The growth rate would depend on the average cycle times of the cells, but the actual eventual tumor size would depend only on the eventual number of stem-like cells. The pattern of growth would depend on how the number of stem-like cells changes over time.

Were the stem-like cells dispersed evenly throughout the three-dimensional mass of a tumor, and were the ratio of symmetrical to asymmetrical divisions stable, the growth of the mass would be exponential since the ratio of stem-like cells to size (K/N) would be the same (Box 1). However, if the stem-like cells are arranged so that their dimensionality is less than three, the relative growth rate of the mass—growth rate divided by total mass size—would slow down as the mass increases in size.

For example, consider a tumor shaped like a solid sphere in which the stem-like cells are on the surface of the sphere only. The volume of the tumor would be proportional to the cube of its diameter (three-dimensionality), whereas the surface would be two-dimensional; that is, it would be proportional to the square of the diameter. As the mass increases in size the ratio of its surface area to its volume, L^2/L^3 drops. Since the stem-like cells are on this surface only, the ratio between the new cell production rate and the mass of the bulk of the tumor, would also drop as the tumor increases in size, leading to an apparent slowing of relative growth (Box 1).

In reality, biological objects are not true sheets or true spheres so the dimensionality of their surfaces is between two and three. Moreover, tumors often have involutions and extrusions, so it may be hard to discern the surface, especially on a microscope slide, which is a two-dimensional cut through the three-dimensional mass of the tumor. The branch-like structure of the tumor vasculature can be thought of as following this surface in a fractal-like pattern with a dimension between two and three.[48] Were a tumor's stem-like cells arranged at its surface only, a tumor's relative growth rate would slow over time as long as that surface had a dimension less than three.

It has been shown that under reasonable conditions of mitosis and apoptosis the above considerations would generate an S-shaped curve that could be indistinguishable from the Gompertzian pattern.[1] Why should these stem-like cells be distributed only, or mostly, at the surface of the tumor mass?

Self-seeding model of malignant growth

The biological basis for self-seeding

A central tenet of cancer biology has been that cancer cells that leave the primary tumor—circulating tumor cells (CTCs)—can then seed metastases in distant organs in a unidirectional process. Data have shown that CTCs can also seed and then colonize their own tumors of origin.[2] CTCs face many barriers for infiltrating and growing in distant organs—tight vascular capillary endothelial walls and an unfamiliar microenvironment among them—so only the most adaptable and hence rare phenotypes could be successful. However, CTCs re-entering the source-tumor itself should encounter a leaky neovasculature and a familiar microenvironment, factors that underlie the tenability of the concept of self-seeding.[1,49,50] This hypothesis was tested empirically and confirmed in various experimental models including breast and colon adenocarcinomas and melanomas.[2] Self-seeding in mice is preferentially mediated by aggressive CTCs in which matrix metalloproteinase (MMP)1, collagenase-1 and the actin cytoskeleton component fascin-1 are expressed. Furthermore, tumor-derived inflammatory cytokines interleukin (IL)-6 and IL-8

act as CTC attractants and promote accelerated tumor growth, angiogenesis, and recruitment of myeloid cells into the stroma (Figure 1). Hence, CTCs alter the microenvironment to make it more supportive of tumor growth. The same biology should be applicable to all mammalian species, including humans, although formal proof in the clinic will require advances in single-cell genomic sequencing and bioinformatics technology (that is, comparing tumor cells from primary and secondary sites), which are currently in progress.

Role of self-seeding in Gompertzian growth

The result of the self-seeding process is that a primary tumor is not one mass, but a conglomerate of contiguous masses. Since the CTCs are coming from the outside of the conglomerate, they would naturally be concentrated at the interface of the conglomerate and the stroma, which is the mathematical definition of a surface. Hence, the growth rate would be proportional to K_t and the growth rate relative to the size of the mass would be proportional to K_t/N_t, which decreases as the number of cells over time gets larger (Box 1). Thus, self-seeding explains the mystery of Gompertzian growth.

The nature of such growth would depend on many factors in addition to the geometric dimension of the growth surface. These factors include those described above: the efficiencies of CTC attraction, matrix dissolution, adhesion, motility, angiogenesis, and stromal recruitment. Conversely, these same factors could influence the dimension magnitude. Additionally, the growth rate would depend on the average cell-cycle time, regulated by a myriad of factors including intrinsic characteristics of the tissue of origin. Hence, the ability to seed is not synonymous with the ability to colonize. Cancer cells must first seed a metastatic site and then, depending on intrinsic factors and the microenvironment, these seeds may multiply such that they then form colonies, and thereby grow into larger masses. This explains the paradox of microscopically detectable cancer cells in organs such as regional lymph nodes and growth to sufficient size to cause local recurrence, or even organ disruption in distant anatomic sites.

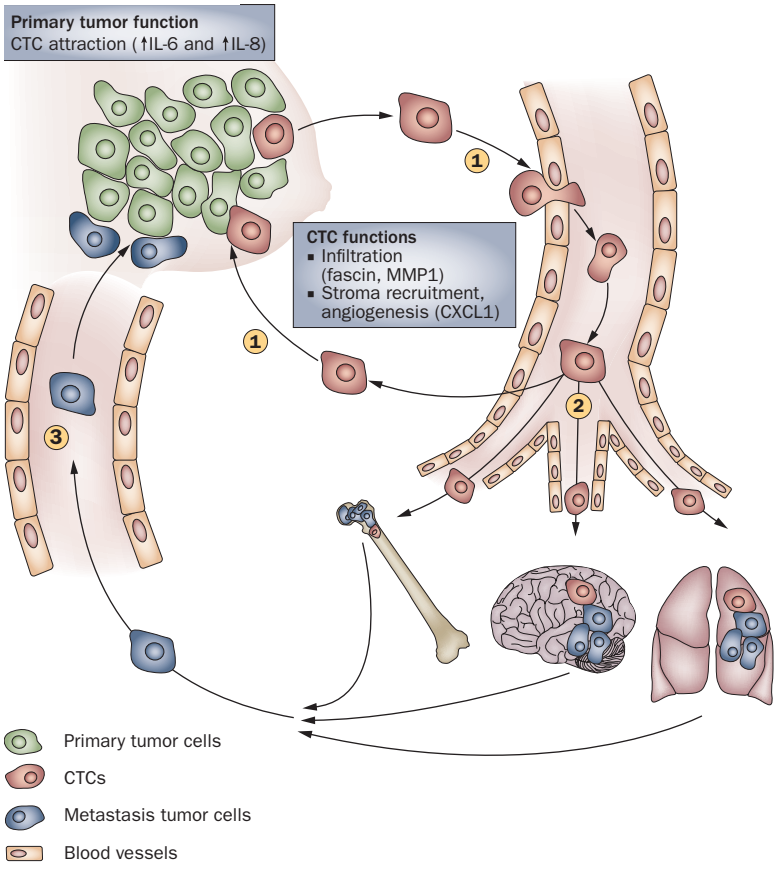

Figure 1 | The concept of self-seeding that explains cancer growth and metastasis. The interaction of the cancer with the surrounding tissue and stromal components is one of the most intriguing aspects of modern cancer biology. The primary tumor mass (depicted on the left of the figure) releases cancer cells. These cells intravasate into the circulation where they follow one of three paths: (1) CTCs seed to the tumor of origin (self-seeding); (2) travel to a secondary distant site such as the lung, brain or the bone; or (3) after extravasating to a distant site, they then return to the primary tumor mass. CTCs alter the microenvironment to make it more supportive of tumor growth. Self-seeding is preferentially mediated by aggressive CTCs in which MMP1, collagenase-1 and the actin cytoskeleton component fascin-1 are expressed. Tumor-derived inflammatory cytokines IL-6 and IL-8 act as CTC attractants, promoting accelerated tumor growth, angiogenesis, and recruitment of myeloid cells into the stroma. Abbreviations: CTC, circulating tumor cell; IL, interleukin; MMP, matrix metalloproteinase.

Self-seeding helps redefine the tumor surface

A critical concept but one that may be difficult to articulate is what is meant by the term 'surface'. To a physicist, engineer, or topologist the term refers to an interface between definable entities, which may well be interdependent but are nevertheless distinct. In this case, the entities are the actual cancer cells and the entire collection of supportive stromal elements—the tumor microenvironment. These include endothelial cells and neovasculature, cells of myeloid origin such as monocytes and macrophages, tumor-associated fibroblasts, and the extracellular matrix. Cancer cells might seem to be admixed or intermingled with such elements, but this does not mean that a 'surface' does not exist. Of course, sometimes the morphologist sees a clear separation of cancer cells and non-cancer cells, often referred to as the 'leading edge' or 'invasive front.' In other cases, the surface may be obscure. For example, the seeding cells (from a contralateral tumor) on microscope slides seem to be admixed with recipient cells, but we know that they define an interface or 'surface'.[2] Here, this surface is defined as between the recipient tumor and its 'outside', which is delineated by contact with the blood supply that carried the seeding cells to it.[2] This 'surface' may not be apparent because biological surfaces are not always smooth. For example, an inflated balloon, which clearly has an outside and an inside, collapses in a tangled heap once the air inside escapes, but it still has the same outside and inside. A morphologist would be hard pressed to decipher what is outside and what is inside a mass of cancer cells, but the reality remains. This is how the appearance of 'admixture' or 'co-mingling' may hide an inside–outside relationship. Furthermore, in the most extreme case, where cancer cells are solitary, unconnected to each other and dispersed throughout an otherwise

normal tissue, the surface of contact between the cancer and its microenvironment would be equal to the sum of the surface areas of all of the cancer cells. But this, of course, is essentially never observed since cancer cells are always found in clumps, although sometimes the clumps are small, only a few cells.

Self-seeding explains certain similarities

The gross molecular similarity between preneoplasia and invasive cancer may reflect that minor changes in self-seeding efficiency could have a major impact on the clinical behavior of cancers. Carcinoma *in situ* could arise in many places in the breast (multifocality) because of intrinsic genomic changes. That is, tissue may seem both morphologically and biologically normal while in fact it is predisposed to malignant transformation by virtue of aberrations at the genomic level. This is termed a 'field defect'.[51] Such cancers may be true cancers but do not seed efficiently, or at all, hence not growing to large sizes by self-seeding and not metastasizing by distant seeding. Since the difference between preneoplasia and neoplasia would be qualitative rather than quantitative, we should expect that the differences at the molecular level should be subtle, as observed.[52] In this respect, the physical structure of the normal breast stroma might be a key aspect of this subtle process.

Self-seeding explains MBD as a risk factor

Self-seeding may promote high dimensionality, which in turn would promote the progression from preneoplasia to neoplasia. This could be the reason why breast density is procarcinogenic; a dense (high dimension) normal collagen matrix could provide a more dangerous starting point for the surface:volume ratio of a transformed cellular state. This would explain the association of breast density with a higher incidence of breast cancer. Indeed, it has been observed that differences in the density of the collagen matrix influence integrin-dependent mechanic transduction and epithelial-cell function and, in turn, promote mammary tumor formation and metastasis.[53]

Stem-cell hypothesis and molecular profiling

Once frank neoplasia ensues, self-seeding would promote tumor growth, by the processes already described, but also lead to the linked characteristics typical of cancers. Because the tumor mass is a conglomerate of small masses, it is by definition disorganized architecturally—that is, anaplastic. The propensity of such a conglomerate to metastasize is simply a reflection of the fact that it is already metastatic in that it grew by 'metastasizing' to itself. Because the tumor of N cells is a conglomerate, with each piece of N_M cells derived from a stem-like seed, the molecular markers that define the cancer are dispersed throughout the mass, hence making molecular profiling feasible.

Redefining concepts

Experiments indicate that seeding is complex and requires the simultaneous function of many proteins, and is thus site specific.[2,54] The gene sets required for self-seeding, for example, lung-seeding, bone-seeding, brain-seeding, overlap to a small extent but are not identical.[45–47,54] Hence, self-seeding capacity should correlate with the capacity to seed to distant sites, but not completely.

The ability of a cancer cell to seed an axillary lymph node should correlate with its ability to seed organs, which should correlate with its ability to seed its primary site; this explains much about clinical observations. Indeed, the self-seeding hypothesis provides a parsimonious approach toward explaining the disparities between existing paradigms and observations in the clinic. Halsted proposed that breast cancer cells need mechanical access to the axilla to seed it; hence, the value of sentinel lymph-node mapping.[12,17] Fisher suggested the breast cancer cell needs a genetic toolkit to allow it to colonize an axillary lymph node should it reach it, but lacking those specific proteins it might instead express proteins that allow it to seed and colonize one of more distant sites.[16] In the existing self-seeding theory, the overlap of gene sets underlying such migratory behavior provides a basis for the correlation between such behaviors and the growth characteristics of the primary cancer.[2]

It is important to appreciate that the ability to seed and the ability to colonize are similarly correlated but are not identical. Consequently, the presence of isolated cells in metastatic sites should not correlate completely with clinically meaningful metastatic behavior. Hence, the presence of unresected axillary involvement is not a guarantee of eventual distant spread, nor is its absence a guarantee of the absence of distant spread.[24] However, cells that seed the organ of origin may be self-selected to be more metastatogenic.[2] Hence, the cells that return to the breast in anatomic locations that are beyond what will be 'clear margins' at lumpectomy are also potential sources of new seeds, again with incomplete correlation.[22] This explains how radiation therapy to the conserved breast could interfere with this process of later seeding, thereby improving prognosis, but not completely, owing to later seeding potential.

The notion that seeds can reside in metastatic sites—including the tissue of origin—and then re-seed other sites at a later time is one with interesting clinical implications. For example, downstream Src signaling may convey survival signals that allow breast cancer cells to reside for decades in a latent form in the bone.[55] Such cells may eventually cause late osseous recurrences, with the possibility of seeding organs other than bones. In addition, metastases from one tumor type to another in the same patient have been observed in clinical samples.[56]

Lastly, although it is most likely that benign tumors are non-seeders or very inefficient seeders, it is possible that in some cases they are excellent self-seeders while inefficient distant seeders. This possibility could also explain the exceptional case where the tumor appears malignant in terms of anaplasia, but is clinically benign in terms of lack of metastatic behavior. Conversely, a tumor may be an inefficient self-seeder but a very efficient distant seeder, such that the primary site may be so tiny as to be occult while many distant metastases ensue. This possibility

might apply to adenocarcinomas of unknown origin or indeed many typical adenocarcinomas of the pancreas.

Self-seeding reshapes our understanding

Perhaps one of the most intriguing aspects of seeding is the observation, highly rational from a biological point of view, that aggressive cancer cells preferentially seed their primary mass of origin. The logic of this is of course that all of the tissue-specific factors that permitted their growth in the first place are concentrated there.[40] Is this why patients with breast cancer so seldom present with stage IV disease? That is, do their CTCs return to the primary breast cancer and the breast itself instead of seeding and colonizing distant sites?

In the early to middle twentieth century, before mastectomy was popular, patients lived with their primary tumors for years, sometimes more than a decade, before they developed metastases.[57] Perhaps these cases were indeed metastatic, but all the metastases were not to distant organs but rather back to the tissue of origin. Since the end of the twentieth century, we have clearly changed the natural history of breast cancer by local control, with many cures but also perhaps the appearance of distant metastases earlier in the time-course of the disease than in the pre-mastectomy period. How the relationship between the resection of macroscopic tumor and the awakening of disseminated but dormant cells manifests requires further study.[58] These speculations also raise the issue of the effects of radiation therapy to the conserved breast following breast-conserving surgery. While such therapy does dramatically reduce the odds of local recurrence, we do not know if CTCs can seed but not colonize an irradiated tumor or tumor-containing breast.

Self-seeding encompasses the major biologic insights and clinical observations. It incorporates the aberrant mobility of cancer cells, the concept of tumor initiating stem-like cells, the role of an evolving stroma, and the importance of the tumor surface and de-emphasizes mitotic aberrations as the sole or even dominant defects. Of greatest consequence, the self-seeding model may perhaps shed light on the long list of enigmas discussed herein.

Self-seeding—therapeutic implications

Before considering some novel implications of the self-seeding model it must be emphasized that some of our current practices might already be consistent with this theory. These include the aforementioned use of radiation therapy following breast-conserving surgery. In addition, some of the medicines in common use to treat breast cancer could have an anti-metastatic effect by interrupting steps in the seeding process. That seeding is so dependent on the recruitment of marrow-derived endothelial precursors and leukocytes—particularly of the monocyte-macrophage family—suggests that disruption of these cells could have a profound effect on metastasis as well as growth.[2,42] Almost all of the cytotoxic agents we now use to treat breast cancer cause leucopenia and other marrow effects, so it is not hard to imagine that they could affect this process by this mechanism.

All new cancer drugs are chosen and developed on the basis of their ability to cause tumor shrinkage in mice. Hence, the methods we use to identify anticancer drugs are fundamentally biased toward an antimitotic effect. Any antimetastatic effect is not evaluated. One of the obvious applications of self-seeding would be to screen existing and new compounds for anti-seeding activity, which should correlate with anti-metastatic effects.[2] This would allow the classification of drugs as anti-mitotic (causing shrinkage) or anti-metastatic (disrupting seeding), permitting the rational design of combination drug therapies (anti-mitotic plus anti-metastatic). We may find that some of the best combinations currently in use may already fit this paradigm.

Of course, in this era of targeted drug therapy it would be rational to use self-seeding laboratory methodology to identify the specific molecules driving the process, develop targeted interventions to disrupt the level or function of these molecules, and thereby design new anti-seeding, anti-metastatic agents. IL-6, IL-8, MMP1, and fascin-1 have already been identified as being implicated, and are obvious candidates.[2] Attention also needs to be paid to the CTCs that are the cancer seeds. These cells must survive in the circulation by switching to anaerobic metabolism, which should make them especially vulnerable to agents targeting this process.[59] Especially attractive might be agents that disrupt many pathways simultaneously such as inhibitors of protein refolding chaperones.[60] It is important to keep in mind that agents that have not passed laboratory drug screens as anti-mitotics might still be useful clinically as anti-metastatic agents, so a fresh look at drug development in this regard might now be justified. New approaches to clinical trial design should also be considered, since usual clinical trial end points—focused on tumor shrinkage—may easily miss anti-metastatic activity.

The most novel suggestion for anticancer therapy to arise from the self-seeding model is ways to kill CTC seeds. We have already discussed radiation therapy in this regard, raising the possibility that an irradiated site could attract CTCs, but the CTCs would not be able to grow by virtue of radiation-altered stroma. Experiments to evaluate the effect and the timing of such radiation exposure are in progress. There is clinical trial evidence that radiation therapy to the breast after systemic adjuvant therapy is more effective than such radiation given before adjuvant chemotherapy in preventing systemic recurrences.[61] The concept for this observation is that the breast is attracting CTCs that are later killed by the radiation therapy, decreasing the burden of these seeds. This would be consistent with observations that patients with soft-tissue sarcomas who undergo re-operation to obtain clean margins actually do better than those with similar histopathologies and clinical stages who are resected to clean margins in one operation.[62] The possible explanation is that the involved margins from the first procedure attracted CTCs that were later removed by the second operation, an effect not possible when a first operation didn't leave involved margins. While these are speculations, they are experimentally evaluable. The

possibility that both CTC attraction and attracted-CTC killing could be augmented by immunological manipulations, such as the use of tumor ablation by interventional radiology or the use of drugs such as anti-CTLA4, is also experimentally verifiable.[63]

Conclusions

The self-seeding model has been confirmed experimentally in mice and is consistent with clinical observations that are otherwise abstruse. Indeed, the concept of self-seeding suggests a new fluid paradigm whereby the progression from primary tumor to metastasis is no longer framed as a unidirectional process. On the contrary, primary tumor growth and metastasis may be understood as multidirectional, balanced between mitotic (primary tumor), seeding (metastasis) and self-seeding processes. In both the laboratory and the clinic, efforts aimed at tipping this balance by altering seeding capacities should be a fertile field for further study. As such, the self-seeding model may be a harbinger for clinical progress.

Review criteria

A formal literature search for this article was not performed. This Review includes a summary of the authors' work and knowledge based on reading the oncology literature. Knowledge gained from regular attendance at conferences, workshops, and other national and international meetings was also included.

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Author contributions

E. Comen and L. Norton both contributed to the writing of this manuscript. E. Comen, L. Norton and J. Massagué contributed equally to researching the data for this article, discussion of the content and reviewing and edited the manuscript before submission and during the editing process.