Cancer Stem Cells, Self-Seeding, and Decremented Exponential Growth: Theoretical and Clinical Implications

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INTRODUCTION

The cancer stem cell hypothesis, for all of its biological intricacy, lends itself to a simple mathematical model with intriguing theoretical and practical implications. Models of this type are neither esoteric nor computation-intensive. Indeed, they can often be expressed in words and images rather than mathematical symbols. This essay will build such a model from basic, experimentally-derived postulates and show how it could explain all of the classical features of epithelial breast cancer: histologic disorganization (dysplasia, anaplasia), rapid growth, large tumor size, angiogenesis, invasion, and metastasis. The model could also explain why anti-mitotic therapy fails to cure most malignant diseases including stage IV breast cancer, and suggests alternative and perhaps superior therapeutic approaches.

Basic postulates

For the purposes of this essay let us express the cancer stem cell hypothesis as a series of postulates, all of which are supported by experimental evidence (Fig. 1) [1–25]

- Within the population of cells that comprise a primary breast tumor there exists a small subset of cells with the special capacity of being able to initiate the growth of a secondary tumor, similar to the primary tumor, should they be separated from the primary tumor and then transplanted [1–6]. These cells are variously called tumor initiating cells or cancer stem cells, this latter term being borrowed from studies of normal organogenesis. Because the word “stem” may convey meanings beyond what is necessary for the purpose of our model, to avoid semantic debate we will here call them type I cells.

- Type I cells are distinguished from the bulk of the cancer cells in the population, which lack the property of being able to regenerate a whole tumor [1,7]. These majority cells are often called differentiated progeny cells. This is a collective term that may include committed progenitor cells, transit cells, transit amplifying cells, and terminally differentiated cells. For simplicity they are here called type II cells.

- It is theoretically possible that a type I cancer cell may arise by genetic change from a normal breast epithelial stem cell or by the dedifferentiation of a type II cell. This is shown in parentheses in Figs 1 and 3 because, while the distinction may have major biological meaning, either pathway would give identical results in the model.
Fig. 1. Possible mitotic fates of type I cells (“cancer stem cells”) and type II cells, the committed progenitors. Since it is not clear if a type I cell can arise by genetic change from a type II cell, this is included in parentheses. Type I cells are in general resistant to drug treatment. Type II cells may be sensitive to such therapy, but may acquire resistance, either randomly or in response to exposure.

- A type II cell has only two possible fates, one of which is mitosis. When it undergoes mitosis, a type II cell proceeds more rapidly than a type I cell [8]. When it divides it produces two type II cells. In some cases, as type II cells progress through a sequence of mitoses they undergo a succession of differentiation stages [9,10]. Type I cells, in contrast, are always undifferentiated.

- When type I cells undergo mitosis, there are two possible outcomes, which might be regulated by cell-environment interactions [7,10–17]. The first, called symmetrical division, is the production of two type I cells. The second, called asymmetrical division, is the production of one type I cell and one type II cell. In both cases, at least one type I cell remains, which is an attribute termed self-renewal [1,7,10].

- The second possible fate of a type II cell – particularly one that is the end product of successive differentiation stages – is programmed cell death [18]. Hence, unlike type I cells, type II cells are limited to a finite number of mitoses.

- Like normal tissue stem cells, type I cancer cells are in general resistant to anti-mitotic drug therapy [18–20]. This may be because they divide relatively slowly, express anti-apoptosis genes, have efficient poison-efflux pumps, or possess other resistance mechanisms conserved in evolution to provide a survival advantage [10,19,21–24].

- Type II cells that are initially sensitive to anti-mitotic therapy may sometimes acquire resistance to such therapy, either spontaneously before exposure to an anti-mitotic drug, or during drug treatment [25].

THE GROWTH PATTERNS OF CANCERS

Our starting point in using these postulates to build a model is the verity that malignant growth is neither linear nor exponential, but follows some pattern between these two extremes (Fig. 2). Linear growth is the type in which, per unit of time, one cell goes to two cells, two cells goes to three, three goes to four and so on. A fixed number of cells – in this example one – is added to the population in a fixed period of time. Clinical cancers have neither been observed to grow this way by direct measurement nor are these patterns logical as regards reasonable time durations. Say, for example, a clinical breast cancer takes two years to grow from one cell to $10^9–10^{10}$ cells (one to ten cubic centimeters of packed cells), a usual size range at diagnosis. By linear growth, were this mass left untreated it would take another two years for it to double in size and yet another two years to triple in size, which is clearly unrealistically slow growth for a cancer.

Exponential growth is the type in which, per unit of time, one cell goes to two cells, two cells goes to four, four goes to eight and so on. A fixed percentage of cells – in this example 100% – is added to the population in a fixed period of time. By this pattern of growth a
Fig. 2. Patterns of growth. Neither pure exponential nor pure linear growth is realistic, as discussed in the text. Decremented exponential growth, of which the familiar Gompetzian curve is but one example, is the most common pattern of tumor growth.

tumor that took two years to grow to a size large enough to be diagnosed at \(10^9-10^{10}\) cells would double in size in 22 to 24 days, triple in size just 13 to 14 days later, and quadruple in size merely 9 to 10 days later. In all but the most extreme hypothetical cases of cancer such growth is unrealistically rapid. Hence, the true growth pattern must lie between the two boundaries of linear and exponential.

The commonly accepted alternative to linear or exponential growth kinetics is some form of decremented exponential growth, as shown in Fig. 2 [26–28]. In these patterns of growth the early part of the curve is close to exponential, but there is a progressive slowing of growth as the population becomes larger. Decremented exponential growth is consistent with much clinical experience. Of the many possible curves of this type, the one that has actually been shown to fit tumor growth curves is the one defined by Benjamin Gompertz in 1825 [27,29,30]. However, the Gompertz curve is just one of an infinite number of possible decremented exponential curves, one of which might fit any given tumor’s growth pattern.

CONSEQUENCES OF THE POSTULATES FOR TUMOR GROWTH

For our model to be realistic, therefore, it must connect the postulates above to decremented exponential growth. Our first question in this regard is what is the relative frequency of the two varieties of type I cell division – symmetric and asymmetric? Clearly, type I symmetrical divisions cannot predominate for two reasons. The first is that because symmetric divisions would double the number of type I cells with each mitotic cycle, type I cells would be plentiful in the population, which they are not [1]. Moreover, a doubling of type I cells with each mitotic cycle would result in exponential growth, a pattern that we have already determined to be unrealistic. Hence, we are left with the conclusion that most type I divisions must be of the asymmetrical kind. This is a point of similarity between type I cancer cells and normal tissue stem cells [8,31].

Figure 3 illustrates the pattern of growth that would result from a process in which all type I divisions are asymmetrical, and each type II cell that is produced by the asymmetrical division of a type I cell subsequently divides a limited number of times.

As shown in Fig. 3, after a few cell cycles the total population size stabilizes – at eight cells in this simplified example, although in reality the number of cells in this stable population would be in the hundreds to account for the known ratio of type I to type II cells found in clinical samples. We may term this group of cells at equilibrium a mature nodule to distinguish it from a new and hence immature nodule that immediately follows a type I cell asymmetrical division, also illustrated in Fig. 3. The mature nodule may take the histological form of a globule, lobule, duct, sheet, column, or more complex form and gene expression pattern depending on the genetic pre-programming of
Fig. 3. Consequences of the basic postulates for the growth of one nodule from one type I cell. The first type I cell might arise by genetic change from a type II cell, but this is not necessary to hypothesize. The immature nodule grows into a mature nodule, which is at an equilibrium size.

its type I cell [32,33]. This may account for the ability of pathologists to identify a cancer’s tissue of origin. It may also explain why breast cancers, for example, fall into discrete categories by hormone receptor and HER2 status (among other variables). It is of note that the growth of each nodule – from stem cell to immature nodule to mature nodule – thus follows a decremented exponential pattern. But it is also of note that a cancer cannot be composed of a single or even a few mature nodules since then the tumor size would be uncharacteristically small, (as, perhaps, in preneoplasia). How then can we explain the central fact that there is a stable in size despite cell turn-over, is by symmetrical divisions of type I cells, each followed by a series of asymmetrical type I divisions and subsequent type II divisions. This is a key deduction: The total growing tumor is not one, whole organized mass, but rather a conglomerate of nodules, some mature and others immature, each with one stem cell in its core.

A MALIGNANT TUMOR AS CONGLOMERATE

The concept of a cancerous mass as a conglomerate cast a new light on all of the cardinal features of cancer [34]. A conglomerate is spatially disorganized; hence, a cancer is anaplastic. Distinct, albeit contiguous nodules would likely induce distinct, albeit densely-packed blood supplies; hence, a cancer is hypervascular. The mass increases in total size by the continuous production of new nodules; hence, it would tend to grow to a large size. In this sense, a breast cancer may be thought of as a mass of thousands of embryonic breasts. Since mature nodules are constant in size, the growth rate of the mass would be proportional to the number of immature nodules at any point of time. Hence, the growth rate (which may be fast or slow, depending on the characteristics of the particular tumor in question) is proportional to the number of symmetrical type I divisions at any point in time.

It is at this point that we discover a paradox, the solution of which may be critical to understanding and possibly eliminating cancer. The mystery is this: If growth rate is proportional to the number of symmetrical type I divisions, and if the number of symmetrical type I divisions is proportional to the number of cells in the population (since the number of cells is proportional to the number of mature nodules, each of which has a type I cell in its core), why is cancer growth not exponential?

Of the several reasonable solutions of this enigma, one especially attractive class of solutions is rooted in elementary geometry [28,34]. The advantage of a geometric, anatomic, physical solution is that it is independent of biological or biochemical subtleties: A chair is a chair no matter what material it is made of as long as that material is sufficiently sturdy. As will be discussed...
below, decremented exponential growth would result if the immature nodules are located at the *periphery* of the conglomerate, at the interface of cancer and stroma, rather than dispersed evenly throughout the mass. Indeed, there are already observations consistent with this supposition. Cells with putative stem cell properties have been found in higher concentrations at the periphery of cancerous masses [12,13,35]. Macrophages, cathepsin production, and other promoters of growth are also found in this location as well [14–17].

There are many ways in which the periphery of a conglomerate mass could be enriched with type I cells. Perhaps symmetrical type I divisions are more likely to occur at the periphery because of a biochemical or physical cancer-stoma interaction [12–17]. Perhaps type I cells that are the products of symmetrical divisions throughout the mass migrate to the periphery, either because of mechanical stresses or because of pro-migratory signals? How this latter possible relates to stem cell anchorage independence in culture would then seem to be an important topic for further elucidation [24].

But another intriguing possibility is that new type I cells relocate to the periphery of the conglomerate following systemic circulation. This has been termed *self-seeding* to recall the way a weed bed grows, not by the massive growth of individual weeds, but by the seeding of new weed plants at the edges of the population [34]. While experiments are still in progress to explore this possibility, let us consider some implications of the hypothesis that may relate rather meaningfully to cancer therapeutics.

**CANCER AS A DISEASE OF SELF-SEEDING**

The concept of cancer as a disease of self-seeding might not only provide a solution to the puzzle of decremented exponential growth, but may bring the phenomena of invasion and metastasis into the conceptual package [34]. This hypothesis is based on the observation that genes responsible for metastasis increased the growth rate of a human-to-murine xenograft in its primary implanted site (mammary fat pad) even though none of the genes are known to primarily regulate mitosis or apoptosis [36,37]. To explain this we hypothesized that the primary site is indeed not just a primary site but also a “metastatic” one. That is, wandering cells might relocate to the primary site just as they might – by using the same biological toolbox – locate to a distant site. That is, just as a weed bed overgrows and destroys a garden even though each weed plant is rather small, so can weeds invade neighboring gardens: The two processes are one and the same.

Most relocating cells would lodge in the primary organ at sites contiguous to the already established mature nodules. This is because the growth signals – from the existing type II cells and perhaps the “cancerized” stroma – would there be in highest concentration. But some post-circulation type I cells might take root in the
primary organ at sites disconnected from the conglomerate mass. The hypothesis explains a common issue in the management of cancer in its site of origin. Even though a breast cancer may be excised with histologically clear margins, one may still find isolated foci of cancer many centimeters away in the same breast [38]. These are – by our hypothesis – metastases to the breast. Hence, breast irradiation is required for optimal local control [39].

If growth and metastasis are linked biologic behaviors, this would explain why large tumor size is associated with a higher probability of metastatic spread [37]. That is, cancers may not metastasize because they are large: They may be large because they “metastasize,” i.e. seed, themselves as well as distant sites. The hypothesis would also explain why the degree of histologic disorganization, the degree of hypervascularity, and the degree of local invasiveness correlate with metastatic behavior: These are all manifestations of the same biologic process – seeding [40]. Indeed, “invasiveness” may be a misnomer since these cells may be relocating circulating cell, adding to the mass from the outside in rather than from the inside out as the word itself implies. Similarly, the terms we use to describe histologic disorganization – hyperplasia, dysplasia, anaplasia – may not be correct, since the defect underlying them could be in cell mobility rather than mitotic control. This reasoning, of course, calls into question our current reliance on anti-mitotic drugs to treat cancer. In doing so we might be treating merely the consequence of the root cause of malignant growth – abnormal type I cell mobility – rather than the cause itself.

SELF-SEEDING AND DECREMENTED EXPONENTIAL GROWTH

We have stated above that the localization of new type I cells and hence immature nodules on the periphery of the growing conglomerate could explain decremented exponential growth: But precisely how? The answer may be found in fundamental geometry (Fig. 5). The periphery (surface area) of a smooth three-dimensional object is proportional to the square of its diameter, while its volume is proportional to the cube of the diameter. As a mass increases in diameter, the ratio of its periphery to its volume decreases. Hence, if the mass is growing largely at its periphery – because that is where the type I cells are found, via migration, production, or circulation – the relative rate of increase in volume of the mass would decrease as it grows larger, proportional to the decreasing ratio of surface area to volume.

But what exactly do we mean by periphery? In reality, the “surface area” is not smooth as with a sphere or other Euclidian object, but rather irregular with invaginations and extrusions, often stellate in shape (Fig. 6). Hence the surface area will not be the square of the mass’s diameter but rather the diameter raised to a power between two and three. In fractal geometry this value is termed a mass dimension [41]. The more irregular the surface, the closer the growth mass dimension will be to three, but in all but the most extreme cases it will be less than three. Yet the dimension of the volume would always be three. Hence, one would always observe a decreasing ratio of surface area to volume as the mass grew larger. Hence, a decremented exponential pattern of growth would result.

Yet – and this is a critically important point – the closer the growth dimension is to three, the more slowly the ratio of surface area to volume will change, so the faster and the larger the mass will grow. This is illustrated in Fig. 6. The larger tumor seen in this figure is more irregular in shape compared with the larger tumor found in Fig. 5. Hence, there is a higher ratio of immature nodules at its periphery to mature nodules in its core. Since, as we have already determined, the growth rate is proportional to the ratio of immature to mature nodules, the geometry of the conglomerate may determine its growth rate as well as the pattern by which that growth rate will change over time. Although beyond the scope of this essay, it merits statement that the growth kinetics of a mass may not only be sensitive to the fractal dimension of the mass, but that the relationship may be non-linear. That is, at a critical number between two and three the growth rate and eventual size of the tumor may change dramatically from slow and small to fast and large. This means that benign and preneoplastic masses could maintain their characteristics (slow and small) despite the accumulation of many genetic changes that affect mass dimension, but then transform suddenly (to fast and large) with just one more genetic-functional change that puts that dimension above a critical threshold.

CLINICAL IMPLICATIONS

The conceptual model described here has a number of distinctive clinical implications in addition to those mentioned above. From clinical experience we have already learned that wide surgical excision, especially
Fig. 5. Ratio of immature to mature nodules for an anatomically regular conglomerate of mature nodules. As the mass grows, the anatomic location of the immature nodules (at the “periphery”) results in a decreasing ratio of new to old nodules, which causes a decreasing relative growth rate. This accounts for the decremented exponential growth of cancers. Exponential growth would occur only if the ratio of immature to mature nodules remained constant during growth.

Fig. 6. Ratio of immature to mature nodules for an anatomically irregular conglomerate of mature nodules. Since the ratio of the “periphery” and volume of such a mass is larger than in the regular case, the ratio of immature to mature nodules will be greater, which accounts for an increased growth rate and eventual mass size.

in combination with regional radiotherapy, is a critical component of a curative therapeutic approach. More unfortunately, we have already learned about the limitations of anti-mitotic drug treatment regarding the cure of advanced breast and other cancers. But the model makes some new points. One is that attempts to kill type I cancer cells may not be the only way to adequately perturb malignant growth [42]. In fact, it may not be the best way, especially with conventional agents, since by virtue of their longer cell-cycle times these cells may be particularly drug-resistant. This is illustrated in Fig. 7. Here the impact of anti-mitotic therapy is profound on the type II cells, with a marked reduction in total population size. However, neither
the asymmetrical nor symmetrical divisions of type I cells are disturbed, so once true drug resistance arises in type I cells, tumor regrowth commences. Indeed, the recurrent situation is worse than the pre-therapy situation. While the tumor burden was contracting in response to treatment, the type I cell population was expanding and seeding. This would explain why, in the modern drug therapy of metastatic breast cancer, after a prolonged period of disease control ends, the cancer is often widely disseminated and hence growths rapidly toward a lethal body burden.

Two alternative approaches would be to perturb symmetrical type I divisions and to reduce the mobility of new type I cells. Both of these would necessitate targeting biochemical processes that have not been in the forefront of anti-cancer drug development. Yet it is possible that we have already touched the surface of this field by beginning to pay attention to anti-angiogenic agents and non-steroidal anti-inflammatory drugs [43, 44]. If an immature nodule cannot mature without its own blood vessel, it cannot contribute to the expansion of the conglomerate mass. Inflammation is all about the mobilization of bone marrow-derived cells, which may share many pathways with the mobility of cancer cells and supporting stromal cells. Intravasation and extravasation, other aspects of the mobility of type I cells, are also potential new therapeutic targets once the gene products permitting such aberrancies are defined.

Yet it is also possible that available agents might have greater utility were they used in a manner consistent with the implications of the new model. The major impact of anti-mitotic therapy on breast cancer has been in the peri-surgical setting [45]. Adjuvant hormonal therapy and chemotherapy have both been shown to improve disease-free and overall survival even though neither approach is effective at eradicating established metastatic disease. Is this because the primary issue in the adjuvant situation is type I cell mobility whereas the primary issues in the stage IV situation – because the disease has already disseminated – are asymmetrical type I cell and type II cell mitoses? Do our anti-cancer drugs perturb new type I cells such that their mobility is impaired? It is in general impossible to answer this question because cell mobility assays are so undeveloped compared with ones that concern cell division. Yet the taxanes – agents of proven effectiveness in the adjuvant as well as the advanced disease settings – are indeed known to impair migration [46]. It is not known if other agents commonly employed in the adjuvant chemotherapy of primary breast cancer have a similar action. What is called for, therefore, is the development of assays that will allow us to choose agents in their proper dose-schedule to optimally and specifically perturb the mobility of type I cancer cells. It is possible – even likely – that the proper dose-schedule needed to kill dividing type II cells is different than the proper dose-schedule needed to maximally inhibit type I cell mobility. Mathematical methods for designing and analyzing pre-clinical experiments in this regard are being developed [47].
Regarding the possibility that we may be able to target symmetrical type I cancer cell divisions, this would be the desired end-product of contemporary research seeking to isolate and characterize promising cancer “stem” cells [42,48–50]. Agents that affect this process, according to the model, would truly be attacking the root cause of cancer rather than what may be a secondary phenomenon: excessive type II cell divisions. Hence, they may not only be more effective in treating cancers, but might be less toxic as well. Furthermore, they may be particularly active in the peri-operative adjuvant and even prevention settings.

CONCLUSION

A simple mathematical model that could explain all of the classical features of epithelial cancer can thus be developed from experimentally-derived postulates. The model itself and its therapeutic implications are all amenable to experimental evaluation, which might confirm its basic tenets or identify weaknesses that could then be addressed constructively. There is no reason to believe that such interplay between conceptual–mathematical models and empirical observations, which has proven useful in many areas of science other than biology, might not contribute to an improved understanding of and control of human breast and other cancers.

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