

*Mathematical Oncology***Paradoxical Dependencies of Tumor Dormancy and Progression on Basic Cell Kinetics**Heiko Enderling,¹ Alexander R.A. Anderson,² Mark A.J. Chaplain,³ Afshin Beheshti,¹ Lynn Hlatky,¹ and Philip Hahnfeldt¹¹Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, Massachusetts;²Integrated Mathematical Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida; and³Division of Mathematics, University of Dundee, Dundee, United Kingdom**Abstract**

Even after a tumor is established, it can early on enter a state of dormancy marked by balanced cell proliferation and cell death. Disturbances to this equilibrium may affect cancer risk, as they may cause the eventual lifetime clinical presentation of a tumor that might otherwise have remained asymptomatic. Previously, we showed that cell death, proliferation, and migration can play a role in shifting this dynamic, making the understanding of their combined influence on tumor development essential. We developed an individual cell-based computer model of the interaction of cancer stem cells and their nonstem progeny to study early tumor dynamics. Simulations of tumor growth show that three basic components of tumor growth—cell proliferation, migration, and death—combine in unexpected ways to control tumor progression and, thus, clinical cancer risk. We show that increased proliferation capacity in nonstem tumor cells and limited cell migration overall lead to space constraints that inhibit proliferation and tumor growth. By contrast, increasing the rate of cell death produces the expected tumor size reduction in the short term, but results ultimately in paradoxical accelerated long-term growth owing to the liberation of cancer stem cells and formation of self-metastases. [Cancer Res 2009;69(22):8814–21]

Major Findings

These findings show not only that proliferation, cell death, and cell migration can influence cancer risk, but that the expected long-term tumor-suppressive influence of cell death, and tumor-promoting influence of extended proliferation, will not always be what unfolds in practice. Interactive dynamics are uncovered in our systems-computational studies that contradict these intuitive projections. This has clear implications in the clinical setting, where chemotherapeutics and radiation are delivered with the expectation of long-term tumor size reduction following cell killing. At the physiological level, our findings also challenge another widely held notion—

that the tumor dormancy observed in almost all adults is necessarily the result of angiogenesis suppression or some other environmentally imposed limitation to growth.

Introduction

As seen in autopsies of adults who have died from noncancer causes, tumors can lie dormant for as long as a lifetime without ever becoming clinically evident (1, 2). Dormant tumors have been identified in many organs such as thyroid, breast, and prostate (3–5), being described as cancer without disease (6). At the same time, it is known that dormant tumors can become malignant and aggressive after undergoing an angiogenic switch (7) and overcoming other genetic and environmental bottlenecks to tumor progression. Understanding how to prevent the conversion of these lesions from harmless dormant nodules into malignant tumors might thus lead to the development of improved cancer treatments. Given the apparent persistence of dormant but continuously cycling lesions in adults as assessed at autopsy, it has been assumed that these lesions contain immortal cells capable of expanding the tumor indefinitely, if only cell death could be averted, e.g., by angiogenic induction or immune suppression. Overlooked in many such investigations of the effect of microenvironment on tumor growth, however, is a pivotal role for basic tumor population kinetics, viewed in a fully interactive setting. Without imposing microenvironmental conditions, we were able to show that the time to significant lesion expansion may vary from well within to well beyond host lifetimes, providing a nonenvironmental explanation for the dormancy phenomenon. The prime determinant of the dormant state proves not to be a limited immortal (stem cell) composition per se, but to a limited ability of stem cells to migrate, coupled, curiously, with a limited death rate and excess replicative potential among the nonstem cells. This finding corroborates recent observations of an inverse dependence between these factors and malignant progression (8, 9).

Until recently, it was believed that a hallmark of the typical cancer cell is unlimited replicative potential and the ability to regenerate tumors (10). In recent years, however, it has been shown for many malignancies that only a small subpopulation of all cancer cells may be immortal and self-renewing (11). Such so-called cancer stem cells (or tumor-initiating cells, tumor-rescuing units, or cancer stem-like cells; refs. 12, 13) have been identified, for example, in leukemia and solid tumors of the colon, breast, and prostate (14–22). Based on these findings, we take into account that, for many if not all tumors, most cancer cells will have a limited replicative capacity ρ_{\max} (12). Here, we investigate the development

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Quick Guide to Model and Assumptions

We use an agent-based model to simulate tumor development. Single cells are considered to be individual objects whose behavior is determined by a fixed set of basic intrinsic cell kinetics and their local environment. At each discrete simulation time point, the cell behavior for all cells is updated and, over time, complex population dynamics emerge.

Basic cell kinetics: Cell proliferation capacity ρ_{\max} , migratory capacity μ , and spontaneous cell death α cooperate to dictate development of the population as a whole.

Assumption 1: The tumor population is intrinsically heterogeneous with cancer stem cells and their nonstem progeny. Stem cells live and are able to divide forever ($\rho_{\max} = \infty$, $\alpha = 0$), and have the ability to produce more such cancer stem cells with probability p_s (symmetrical division). Progeny nonstem cells, in contrast, have a limited proliferation capacity and can also spontaneously die ($\rho = \rho_{\max}$, $\alpha > 0$).

Assumption 2: Cells compete with each other for available space. Cells completely surrounded by other cells are considered to be spatially inhibited and sent into quiescence.

divide symmetrically to produce another cancer stem cell. We then explore more generally how intrinsic tumor kinetics can keep tumors in dormancy or promote certain morphologic features of tumor growth often attributed to higher order tumor microenvironment interactions.

Materials and Methods

We have implemented a cellular automaton model (23–33) based on rules for independent cells in response to their local environment (see ref. 34 for a review of similar model approaches). Cells are defined as independent agents that

of tumors with heterogeneous cell proliferation capacities and discuss implications for cancer treatment. As a first case, we assume that the first cancer cell is a progenitor or transformed differentiated cell with a limited life span. We then investigate the population dynamics if that first cancer cell is indeed clonogenic, i.e., has unlimited replicative potential $\rho_{\max} = \infty$. Finally, we compare those populations with the development of a population arising from a true cancer stem cell, i.e., a cell with unlimited replicative potential and the additional ability to occasionally

have intrinsic properties. At discrete time points, the age of all cells is advanced in a random order, and depending on the states of other cells and the local environment the state of a given cell is updated. We found that a variety of self-organizing population morphologies arise from stochastic simulations of the space-limited expansion of descendants of single independent cells possessing differing capacities for replication and renewal.

The domain under consideration is a two-dimensional lattice of $3,500 \times 3,500 \mu\text{m}$, divided in 350×350 lattice points of $100 \mu\text{m}^2$ each. A lattice point can host at the most one cell at any time. Cells are considered as individual

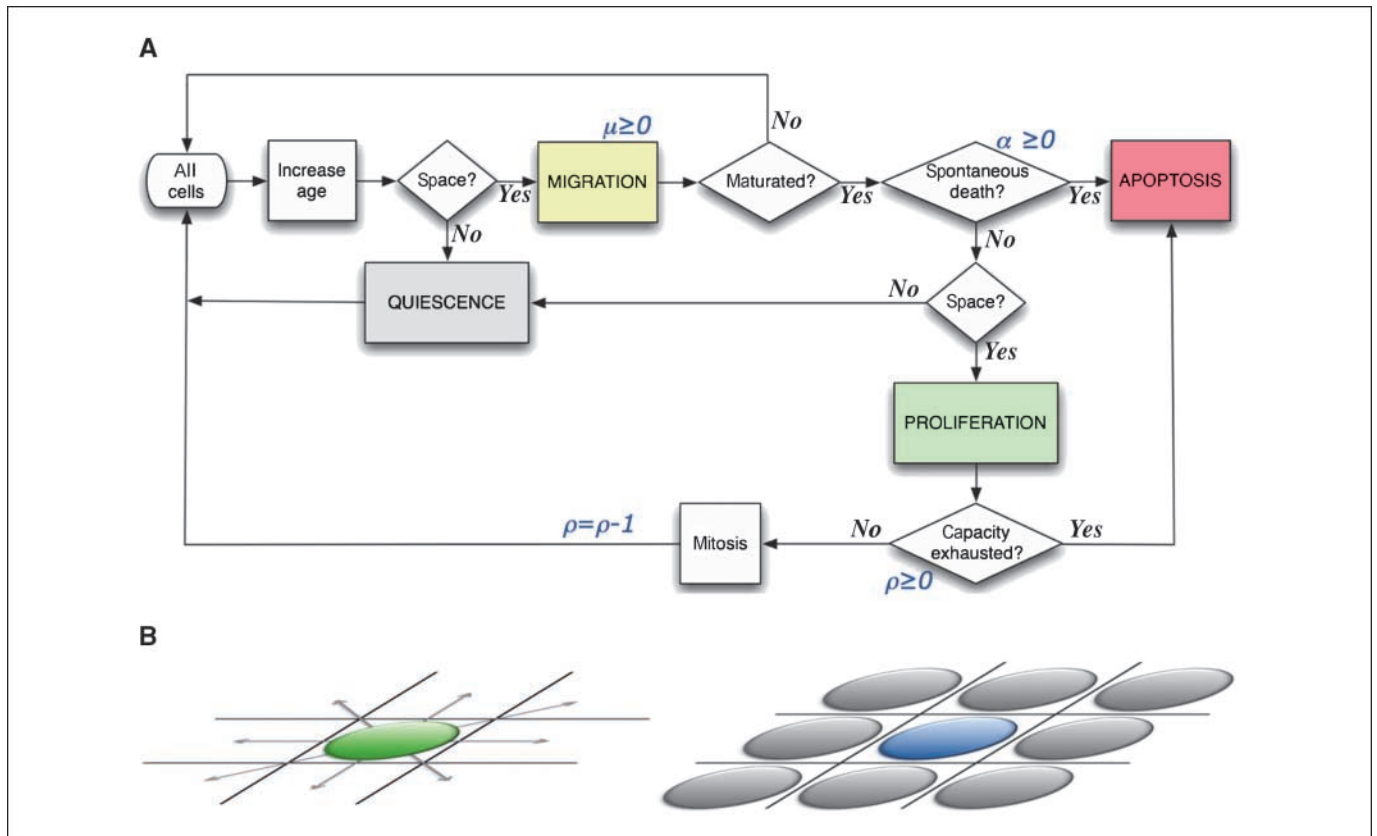


Figure 1. A, cell life cycle scheme. At each time step, the cell age increases. The cell will rest in quiescence if there is insufficient space. If there is space the cell can migrate, and if there is sufficient space for the cell to divide, it will proceed into mitosis. In the event of a spontaneous death decision or if the proliferation capacity is exhausted, the cell will be removed; otherwise, it will produce a daughter cell. B, schematic realization of competition for space. A cell with vacant neighboring lattice points has sufficient space to migrate and proliferate (left). If a cell is completely surrounded by other cells, it is inhibited and sent into quiescence (right).

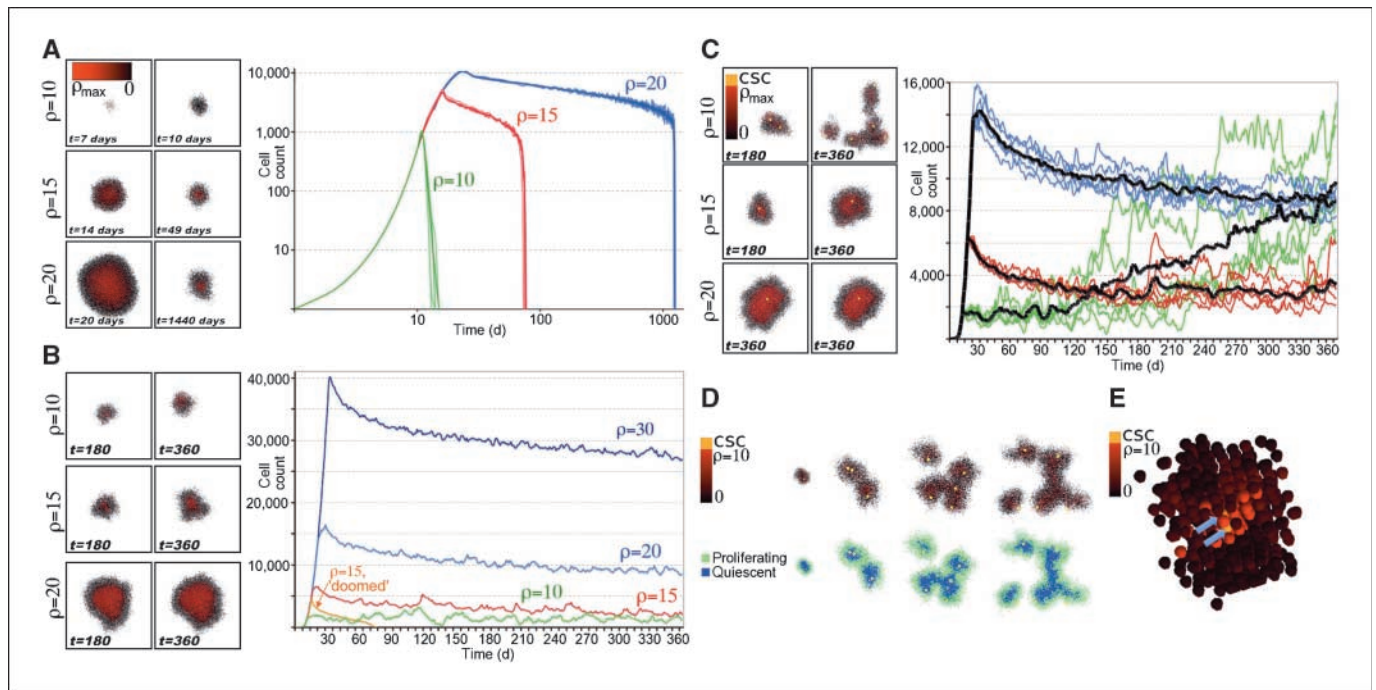


Figure 2. *A*, nonclonogenic tumor development. Simulations of tumor development from a single cell with limited replicative potential $\rho = 10$, $\rho = 15$, and $\rho = 20$ divisions (individual cell proliferation capacity is color coded with red and black representing $\rho = \rho_{\max}$ and $\rho = 0$). Initial exponential growth is followed by a steady decrease in cell number over time. All simulations show oscillations in cell number due to cells death, cell proliferation, and transition between quiescent and active cell compartments. *B*, clonogenic tumor development. Simulation of tumor development for $t = 365$ d from a single cell with unlimited replicative potential. Progeny proliferation capacities are $\rho = 10$ (green), $\rho = 15$ (red), $\rho = 20$ (blue), and $\rho = 30$ (dark blue) divisions (individual cell proliferation capacity in the simulations is color coded with red and black representing $\rho = \rho_{\max}$ and $\rho = 0$). After an initial exponential growth, tumor sizes oscillate around a pseudo-steady state. For comparison, the “doomed” population (i.e., no unlimited replicative potential) for $\rho = 15$ that vanishes after 71 d is shown in orange (refer to red plot, *A*). *C*, tumor development from a true stem cell (yellow) with symmetrical division probability $p_s = 1\%$. All tumors feature an initial exponential growth followed by oscillations around a pseudo-steady state. Only in the population with low progeny proliferation ratio of $\rho = 10$ is the stem cell quickly exposed to space and hence proliferates more often subject to symmetrical division. The resulting population features on average a stem cell pool of 6.8 ± 3.1 cells after $t = 360$ d, and the tumor is a conglomerate of individual cell clusters. *D*, self-metastatic tumor morphologic evolution. Spatial distribution of proliferation capacity and proliferation/quiescence state within a growing tumor at times $t = 30, 150, 250, 365$ d. *E*, high resolution of a representative three-dimensional simulation of the initial tumor cluster after the cancer stem cell (yellow) has divided (arrows).

entities with a cell cycle, maturation age of 1 d, migration potential of $\mu = 0$ to $0.00635 \text{ mm h}^{-1}$ (≈ 1 cell width/96 min; ref. 35), and a replication capacity of $\rho = (0, 1, \dots, \rho_{\max})$. A replication capacity of ρ_{\max} means that one cell can divide ρ_{\max} times, producing potentially $2^{\rho_{\max}}$ cells, before undergoing cell death. In the case of a cancer stem cell, ρ_{\max} is infinite. Algorithmically, at discrete time intervals $\Delta t = 96$ min (15 times per day), the age of each cell gets incremented, and if space is available, the cell can migrate, or (if the maturation age has also been reached) divide, reducing ρ by one (Fig. 1A).

We are allowing the tumor to grow and expand in “empty” space to study intrinsic tumor dynamics without environmental challenges. The goal is to fully understand how individual cancer cell kinetics contribute to intrinsic tumor growth. This is being done in part to test whether features of tumor growth attributed to environmental factors, e.g., angiogenesis and immunity, might actually have a basis in intrinsic properties shared by the tumor cells themselves when studied in a fully interactive setting. Accordingly, we consider early avascular tumor growth with cell numbers below the oxygen diffusion threshold. However, cancer cells at all stages need to grow to successfully divide, and for expansion to occur, several studies have shown that sufficient space must be available (36–40). At its basis is pressure from adjacent cells (39, 40), which limits the ability of tumor cells to “push” their neighbors to enable division except at the tumor periphery (38), along with an ability of cells to migrate, a distinguishing characteristic of cancer cells not captured in, e.g., preneoplastic modeling (36). For our purposes, we therefore define a cell as being inhibited (quiescent), unable to either divide or migrate, when other cells occupy all adjacent lattice points (Fig. 1B).

Results

Tumor growth from a cell without clonogenic potential. We first considered the case of the growth of a single cancer cell that has limited replicative capacity (Fig. 2A). We initialized a single cancer cell in the center of our computational domain with sample proliferation capacities of $\rho_{\max} = 10$, $\rho_{\max} = 15$, and $\rho_{\max} = 20$ cell divisions; a migration rate of $\mu = 15$ cell widths per day and no random cell death (i.e., $\alpha = 0$); and ran five independent simulations for each proliferation capacity. Initial exponential growth made possible by migration gave way to space-limited growth, keeping the cluster sizes well below the potential $2^{\rho_{\max}}$ cells in each case. At the same time, larger ρ_{\max} values corresponded to larger final cluster sizes. On average, the population with $\rho_{\max} = 10$ reached a maximum cell number ($n = 942$) after 11 days and disappeared after 15 days. For $\rho_{\max} = 15$, the population attained an average maximum of $n = 4,838$ cells at $t = 16$ days. Despite oscillations in cell numbers due to previously quiescent cells becoming active again after adjacent cells die, the number of cells eventually decreased until the population vanished after ~ 71 days on average. Finally, for a proliferation capacity of $\rho_{\max} = 20$, the population size peaked after 28 days at $n = 10,457$ cells. This population, although fated to die out, persisted for 1,225 days (almost 3.5 years) with an average population of 2,000 to 4,000 cells for most of that time. Further increases of the initial proliferation

capacity to $\rho_{\max} = 25, 30,$ and 50 divisions yielded average maximum cell numbers of $24,000, 35,000,$ and $117,000,$ and to nonmalignant cell clusters that persisted for many years.

Tumor growth from a clonogenic cell. Our simulations showed that nonclonogenic cancer cells are unable to form tumors of any appreciable size, despite the large potential sizes predicted by ρ_{\max} values. For a tumor to exhibit substantial growth and persist without extinction, it needs a cell with unlimited replicative potential that can constantly repopulate the decaying cluster (Fig. 2B). A cell with limited replicative potential may acquire unlimited replicative potential in different plausible ways: (a) via a point mutation that inhibits telomere shortening (41), (b) via dedifferentiation (42), or (c) via fusion of the cancer cell with a normal stem cell (43). If we assume that a single clonogenic cancer cell has unlimited replicative potential ($\rho_{\max} = \infty$) and all daughter cells have only finite replicative potential, then similar to the simulations just described, cell clusters develop with initially exponentially increasing cell number. However, the final density- and proliferation capacity-dependent growth limitations described earlier can now be countered by the opportunistic proliferation of the clonogenic cell into space as it occasionally becomes available. As a result, the size of the cancer cell cluster comes to oscillate around a certain final size. Figure 2B shows representative simulations of tumor formation from single cells with migratory capacity $\mu = 15,$ and unlimited replicative potential and different progeny proliferation capacities $\rho = 10, 15, 20,$ and $30.$ With increasing progeny proliferation capacity, the tumor sizes increased—yet no tumors expanded continuously to constitute a clinical threat.

Tumor growth from a cancer stem cell. The common understanding is that single cells with clonogenic potential can give rise to a full-sized tumor, or repopulate the tumor if they survive treatment. Our simulations, however, show that a single cell with unlimited replicative potential is not sufficient to explain exponentially growing tumors up through the size of clinical detection. To see such tumors, we propose that multiple clonogenic cells must exist that each form individual clusters of cancer cells, that aggregate to form one large tumor. Such subpopulations of potent cells could arise either by constantly recruiting new clonogenic cells by one of the mechanisms described previously, or by the symmetrical division of clonogenic cells, creating two clonogenic cells from one (44, 45). The latter mechanism would classify the clonogenic cells as true cancer stem cells. In our next study, we assumed the existence of true cancer stem cells that can divide symmetrically with a certain probability of $p_s = 0.01$ roughly approximating the stem cell frequency observed in solid tumors (46). Thus, whenever a cancer stem cell divides, it has a 1% chance of forming a cancer stem cell and a 99% chance of asymmetrically dividing to produce a stem cell and a differentiated cell. We started the simulation with a true cancer stem cell and assumed as in the first study that all cells have migratory capacity of $\mu = 15$ and the nonstem cells have proliferation capacities of $\rho = 10, 15,$ and 20 (Fig. 2C). In all simulations, cells initially produced many daughter cells that went on to divide freely, leading again to an initially exponentially growing population. As before, the clusters that developed quickly occupied the space around cancer stem cells, inhibiting their further division. These cells remained quiescent until they were exposed to space once again (Fig. 2D). In early development, therefore, events paralleled the cases where there were either no immortal cells or immortal self-renewing cells, with increased progeny proliferation capacities resulting in larger cell clusters. At longer time scales (on the order of months), however, the picture changed markedly and

counterintuitively. When nonstem cells had lower proliferation capacities, space was made available earlier on for stem cells to divide and produce both nonstem and stem cells. In this way, shorter proliferation capacities actually favored more stem cell production and better long-term growth rates. In our simulations for $t = 365$ days, tumors arising from a single cancer stem cell with $\rho_{\max} = 10$ developed a cancer stem cell pool of 6.8 ± 3.1 cells on average (averaged over five independent runs). This cancer stem cell population formed individual clusters of cells, resulting in a tumor growing almost 10 times larger than the size expected in the absence of the ability of cancer stem cells to symmetrically divide (see Fig. 2B). The pattern of the resulting tumor growth can be described as a conglomeration of individual tumors, an idea that has been proposed previously (47, 48). In contrast, tumors forming from stem cells with more potent progeny ($\rho = 15, 20$) could not initiate significant growth, as the stem cells were less often exposed to space and hence divided less often, either asymmetrically or symmetrically. Instead of the direct variation between both short- and long-term tumor size and proliferation capacity observed in the absence of self-replicating stem cells, we observed here a direct short-term dependence, a *U-shaped* intermediate-term dependence, and a long-term inverse dependence. In the intermediate term ($t = 365$ days), the $\rho = 15$ group showed lower tumor growth than $\rho = 10,$ with $\rho = 20$ settling somewhere between these two. In only one of five stochastic simulations, the tumors arising from stem cell progeny with proliferation capacity of $\rho = 15$ divided symmetrically once, increasing the stem cell pool size and the total cell count. An example of symmetrical stem cell division in a three-dimensional simulation is shown in Fig. 2E. Both stem cells are in the core of the tumor cluster with a radial proliferation capacity gradient. In none of the simulations of tumors arising from progeny with proliferation capacity of $\rho = 20$ was symmetrical division seen within the first $t = 365$ days. At later times, with $\rho = 15$ and $\rho = 20$ the tumors form conglomerates of individual tumors as well, with overall tumor cell numbers inversely depending on progeny proliferation capacity ρ_{\max} (48).

Increased apoptosis and tumor progression. Thus far, we have discussed tumor formation from cells with limited replicative potential, unlimited replicative potential, and true cancer stem cells. Our simulations suggest that a true cancer stem cell is necessary to give rise to a malignant tumor, yet may not be sufficient, as the evolving cell cluster can inhibit the proliferation of the potent stem cell and thus prevent malignant behavior in biologically and clinically observed time frames (especially for larger proliferation capacities ρ_{\max} than considered here). This observation is in line with previous findings that tumor growth can indeed be intrinsically inhibited by its own mass (49). In our simulations, only short-lived progeny (i.e., $\rho = 10$) die fast enough to vacate space for the cancer stem cell to proliferate into. We speculated that a spontaneous rate of apoptosis (50, 51) or mitotic cell death might have an analogous effect, freeing space for higher stem cell production and overall population turnover. Increased tumor size with cell death was in fact observed in the long term—a complete reversal of the direct suppression of tumor growth with cell death observed (and expected) in the short term (Fig. 3). To uncover this dependence, we simulated tumor development for different spontaneous cell death rates $\alpha = 0, 0.05, 0.1, 0.15, 0.25,$ and 0.35 (i.e., 0%–35%) for constant $\rho = 15$ and $\mu = 5.$ We initialized each simulation again with one true cancer stem cell and stopped the simulation after $t = 35$ months (7 years) or when the tumor reached confluence within the domain. In the first few simulated months, tumor

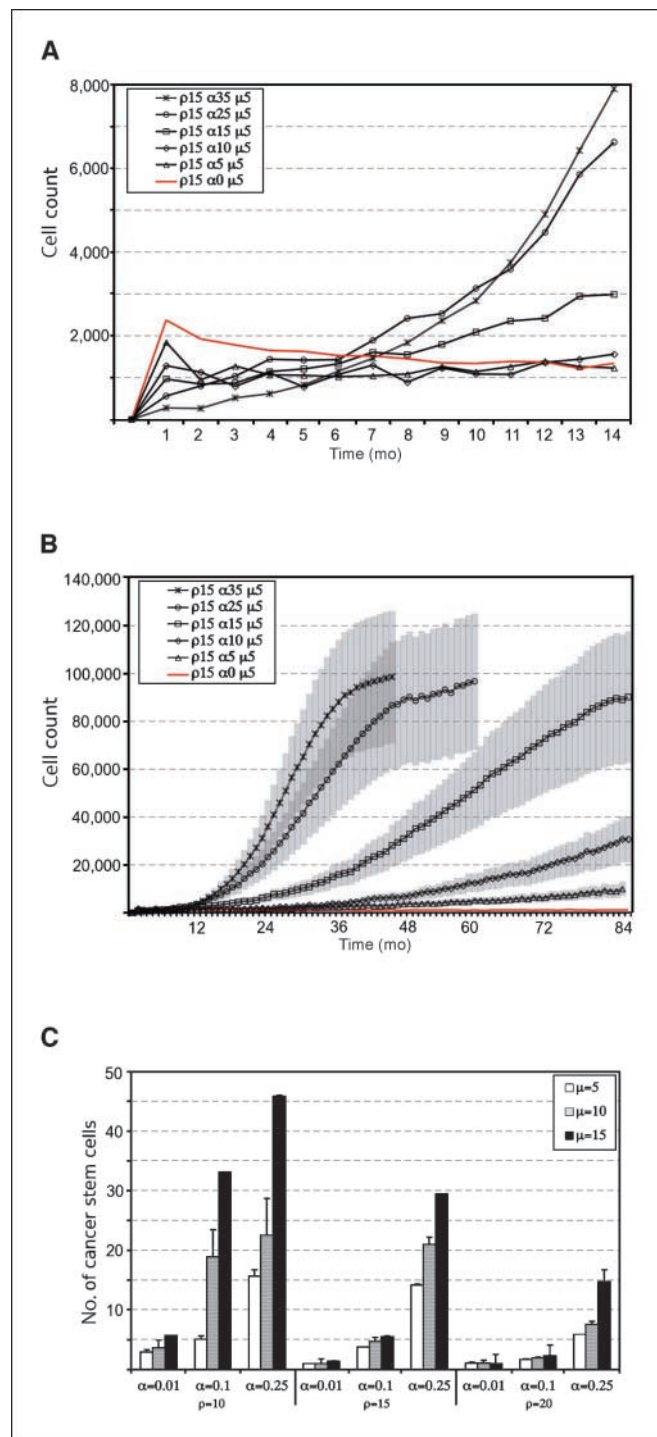


Figure 3. Paradoxical role of cell death. *A*, spontaneous cell death–dependent tumor growth for constant progeny proliferation capacity of $\rho = 15$ and migration of $\mu = 5$. Initially increased spontaneous death rates reduce tumor cell number accordingly (more cell death results in smaller tumor numbers), but promote tumor growth (more cell death results in larger tumor numbers) after 6 to 14 mo. *B*, paradoxical spontaneous cell death effects are a long-time phenomenon (here shown as 7 y). Shown are averages of 10 independent simulations with SEMs. *C*, parameter-dependent stem cell numbers after simulated 365 d. Symmetrical cancer stem cell division is dependent on spontaneous cell death rate α modulated by progeny proliferation capacity ρ and migration speed μ . With increasing cell death ($\alpha = 0.01, 0.1, 0.25$) the initiating cancer stem cell divides more often resulting in a larger cancer stem cell number. This effect is promoted with increasing migration rate ($\mu = 5, 10, 15$) and decreasing progeny proliferation capacity ($\rho = 20, 15, 10$). Columns, mean 10 independent simulations; bars, SEM.

sizes were smaller in direct relation to the amount of imposed cell killing. However, after ~ 6 months, the trend started to reverse for the $\alpha = 0.15, 0.20,$ and 0.35 groups, and after approximately 12 to 15 months for the $\alpha = 0.05$ and 0.10 groups, until a complete trend of accelerated tumor growth with cell killing was realized (Fig. 3*B*). Enabling the eventual reversal of growth kinetics with cell death was the ability of cells, and tumor stem cells in particular, to migrate and replicate in the space made available by cell death within the growing tumor mass. To support this hypothesis, we examined the cancer stem cell pool in a tumor arising from a single cancer stem cell as a function of progeny proliferation capacity ($\rho = 10, 15,$ and 20), spontaneous progeny cell death rate ($\alpha = 0.01, 0.1,$ and 0.25), and cell migration rate ($\mu = 5, 10,$ and 15 cell widths/day). For each of the $3^3 = 27$ combinations of parameters, we ran 10 independent simulations and monitored the number of cancer stem cells in the population after $t = 365$ days. As expected, the number of cancer stem cells increased as the migration speed μ increased (with α and ρ fixed), but paradoxically, also as the rate of spontaneous cell death α increased (with μ and ρ fixed). Equally surprisingly, as the progeny proliferation capacity ρ increased (with μ and α fixed), the number of cancer stem cells in the tumor decreased (Fig. 3*C*). We conclude that density-derived inhibition of tumor growth may be overcome and malignant growth permitted only when there is a sufficiently high rate of cell migration and cell death or a sufficiently low proliferation rate—associations that clearly challenge basic intuition. More generally, many of the common features of cell growth, including initial formation of gaps in tumor clones, the filling of those gaps, and growth by self-seeding of clones (Fig. 4) can be readily explained by simple cell kinetics, often with unexpected results. These include the elaborate colony morphologies, e.g., holoclones (round colony outline, densely packed cells), meroclones (densely packed cells in the core, separated cells at the periphery), and paraclones (few cells in contact, colony largely scattered), which have previously been described (52).

Discussion

We have presented a cellular automaton model of tumor formation from cells with limited replicative potential, unlimited replicative potential, and true cancer stem cells. Our simulations show that tumors developing from cancer cells without clonogenic potential will inevitably die out, and cancer stem cells (or tumor-initiating cells) are necessary for malignant tumor growth. Assuming the evolving tumor possesses a true cancer stem cell, manifestation of malignant disease can still be suppressed by limitations on cancer stem cell proliferation posed by cell crowding in the dense tumor core. Our simulations suggest that tumor populations devoid of stem cells or developed from cancer stem cells could still persist as long-term dormant lesions, in line with recent autopsy evidence showing a high prevalence of small asymptomatic, dormant tumor lesions in most adults (3). By contrast, a high rate of spontaneous cell death modulated by cell migration can provide tumor dynamics that enable sufficient stem cell divisions to enrich the stem cell pool and drive malignant expansion (53). Our simulations go on to show spontaneous cell death can reduce the number of cancer cells in the short run, but in the long run, liberate cancer stem cells, thereby promoting tumor progression. We therefore conclude that high rates of spontaneous cell death in tumors may not be disadvantageous for tumor growth, but in fact may facilitate tumor progression up to a malignant disease.

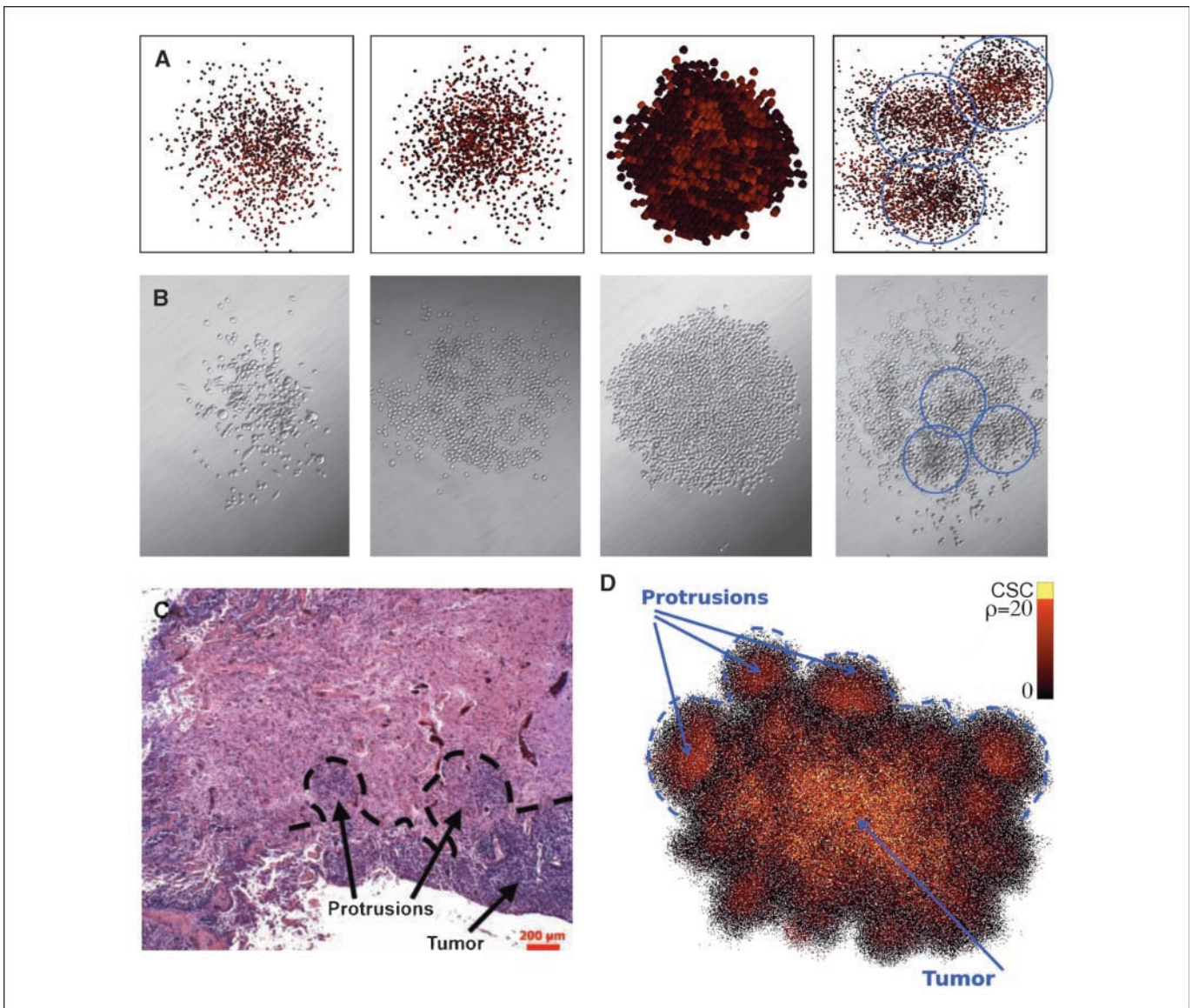


Figure 4. Cell cluster and tumor morphologies. *A*, all the common features of cell growth, including initial formation of gaps in tumor clones, the filling of those gaps, and growth by self-seeding of clones can readily be explained by basic cell kinetics. *B*, *in vitro* cell colonies of tumorigenically transformed murine lung fibroblasts arising from a single-plated cell per well displaying migration-dependent clusterings. *C*, histology section from a glioblastoma reveals protruding cell fronts into an area of nevascularized host tissue. [Reprinted with permission from Bearer and colleagues (60)]. *D*, irregular tumor morphology and invasive protrusion develop from the interplay of basic intrinsic cell kinetics without environmental stimulation. $\rho_{\max} = 20$, $\mu = 15$, $\alpha = 1\%$, $p_s = 0.25$, $n = 100,000$ cells.

This observation challenges a fundamental assumption in radiotherapy and chemotherapy—that long-term tumor growth may be efficiently suppressed by up-front cell killing. In doing so, our results have a number of immediate therapeutic translations. One is that the phenomenon of accelerated recurrence following therapy can be explained by the opportunistic proliferation of quiescent tumor cells into space made available by the initial cell killing. The short-term positive tumor response is followed by a rate of regrowth that exceeds that of the original tumor. Importantly, this treatment-recovery cycle favors the creation of new stem cells produced by the symmetrical division of previously quiescent stem cells, as cancer stem cells are generally more resistant to radiation (54) and chemotherapy (55) than their nonstem counterparts, and because cancer stem cells persist, once created. Analogously, when random cell death among nonstem cells is increased (Fig. 3), an increase in

stem cell count is noted. As the tumor returns to its pretreatment size, then, a tumor more refractory to treatment is expected. These results support previous observations that a high rate of apoptosis in cancer cells may actually promote tumor progression (8), and that upregulation of antiapoptotic factors may act to suppress tumor progression and improve prognosis (9). Our observations complement explanations for why cancer treatments that induce cell death in cancer cells but fail to accomplish eradication can lead to relapse and an even larger cancer cell burden (56).

A second therapeutic implication relates to the nature of the tumor resulting from treatment. As cancer stem cell status has been directly linked with metastatic potential (57), the larger stem cell fraction predicted equates to a more aggressive and metastatic phenotype overall, carrying with it a presumably poorer prognosis. The changed character of the population as a whole accords with

paradoxical prognostic findings surrounding higher apoptosis, including a reduced net tumor doubling time (58). We have attributed these responses not to the cell killing per se, but to the vacating of the positions the cells occupy. It stands to reason that if therapies could be directed to disabling proliferation but not at the expense of cell attrition, better outcomes might be expected. Having said this, te Poele and colleagues (59) point out that some chemotherapeutic agents can reawaken the senescence response in some p53-expressing tumors, a possibility that has exciting treatment implications. Senescent cells would comprise an obstacle to the expansion of proliferation-competent stem and nonstem cells. This could inhibit the accelerated repopulation found to occur when targeted cells are removed from the system. As this would induce stable disease, the relative lack of appreciation of this effect, and specifically its connection to p53 status, may lie in the current clinical practice of categorizing stable disease with nonresponse.

Finally, that the accelerated response accords with clinical observation lends great support to the cancer stem cell hypothesis at the core of our modeling. As stated, treatment has the effect of disturbing an equilibrium between cancer stem cells and nonstem cells, making for an altered tumor character that persists during and even after therapeutic recovery. This is contrasted with the alternative concept of a tumor comprised only of clonogens capable of replenishing the tumor. In this latter case, the intrinsically homogeneous stem cell composition does not permit shifts of subpopulation composition following dosing. As a result, suppression and recovery cannot yield qualitatively different population kinetics. In this way, our results are consistent with the cancer stem cell hypothesis and, along with it, the caution that the "typical" (non-stem) cancer cell profiled for molecular targeting may not represent the best cancer target.

The model driving these conclusions is based on a minimal set of independent parameters that determine intrinsic tumor dynam-

ics. Tracking these parameters in an agent-based framework, we derived conclusions as to how tumor inhibition can be achieved, and how perturbation of intrinsic tumor parameters can alter tumor growth morphology. These studies augment those of Bearer and colleagues (60) who have noted a connection between an invasive "protrusion" feature of tumors and certain characteristics of the environment. It is noteworthy that we were able to observe similar protrusion effects in the absence of explicit environmental considerations (refer to Fig. 4D), attesting to the potential value of a concerted study of basic cell kinetics for refining our understanding of cause and effect in tumor development. Such valuable insights are often unavailable in complex models where extrinsic factors interact with the tumor and alter cancer cell proliferation and migration, or even induce cell death. Future development of the model is straightforward, and introduction of rules for interactions with the microenvironment and the host immune system will give insights into how different treatment strategies act to provide or fail to provide tumor control.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285:1182-6.
- Hahnfeldt P, Panigrahy D, Folkman J, Hlatky L. Tumor development under angiogenic signaling: a dynamical theory of tumor growth, treatment response, and post-vascular dormancy. *Cancer Res* 1999;59:4770-5.
- Black WC, Welch HG. Advances in diagnostic imaging and overestimations of disease prevalence and the benefits of therapy. *N Engl J Med* 1993;328:1237-43.
- Sweeney E. Dormant cells in columnar cell carcinoma of the thyroid. *Hum Pathol* 1995;26:691-3.
- Neves-E-Castro M. Why do some breast cancer cells remain dormant? *Gynecol Endocrinol* 2006;22:190-7.
- Folkman J, Kalluri R. Cancer without disease. *Nature* 2004;427:787.
- Abdollahi A, Schwager C, Kleeff J, et al. Transcriptional network governing the angiogenic switch in human pancreatic cancer. *Proc Natl Acad Sci U S A* 2007;104:12890-5.
- Wodarz D, Komarova N. Can loss of apoptosis protect against cancer. *Trends Genet* 2007;23:232-7.
- Gurova KV, Gudkov AV. Paradoxical role of apoptosis in tumor progression. *J Cell Biochem* 2003;88:128-137.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57-70.
- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003;100:3983-8.
- Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat Rev Cancer* 2008;8:545-54.
- Price ND, Foltz G, Madan A, Hood L, Tian Q. Systems biology and cancer stem cells. *J Cell Mol Med* 2008;12:97-110.
- le Viseur C, Hotfilder M, Bomken S, et al. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. *Cancer Cell* 2008;14:47-58.
- Kavalerchik E, Goff D, Jamieson CHM. Chronic myeloid leukemia stem cells. *J Clin Oncol* 2008;26:2911-5.
- Boman BM, Huang E. Human colon cancer stem cells: a new paradigm in gastrointestinal oncology. *J Clin Oncol* 2008;26:2828-38.
- Pohl A, Lurje G, Kahn M, Lenz HJ. Stem cells in colon cancer. *Clin Colorectal Cancer* 2008;7:92-8.
- Kakarala M, Wicha MS. Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. *J Clin Oncol* 2008;26:2813-20.
- Rody A, Holtrich U, Kaufmann M. "Stem cell like" breast cancers-A model for the identification of new prognostic/predictive markers in endocrine responsive breast cancer exemplified by Plexin B1. *Eur J Obstet Gynecol Reprod Biol* 2008;139:11-5.
- Maitland NJ, Collins AT. Prostate cancer stem cells: a new target for therapy. *J Clin Oncol* 2008;26:2862-70.
- Kelly K, Yin JJ. Prostate cancer and metastasis initiating stem cells. *Cell Res* 2008;18:528-37.
- Michor F. Chronic myeloid leukemia blast crisis arises from progenitors. *Stem Cells* 2007;25:1114-8.
- Alarcon T, Byrne HM, Maini PK. A cellular automaton model for tumour growth in inhomogeneous environment. *J Theor Biol* 2003;225:257-74.
- Anderson ARA. A hybrid mathematical model of solid tumour invasion: the importance of cell adhesion. *Math Med Biol* 2005;22:163-86.
- Anderson ARA, Weaver AM, Cummings PT, Quaranta V. Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. *Cell* 2006;127:905-15.
- Betteridge R, Owen MR, Byrne HM, Alarcon T, Maini PK. The impact of cell crowding and active cell movement on vascular tumour growth. *Networks and heterogeneous media* 2006;1:515-35.
- Byrne HM, Owen MR, Alarcon T, Murphy JR, Maini PK. Modelling the response of vascular tumours to chemotherapy: a multiscale approach. *Math Model Meth Appl Sci* 2006;16:1219-41.
- Chaplain MAJ, Graziano L, Preziosi L. Mathematical modelling of the loss of tissue compression responsiveness and its role in solid tumour development. *Math Med Biol* 2006;23:197-229.
- Enderling H, Alexander NA, Clarke E, et al. Dependence of invadopodia function on collagen fiber spacing and crosslinking: computational modeling and experimental evidence. *Biophys J* 2008;95:2203-18.
- Enderling H, Park D, Hlatky L, Hahnfeldt P. The importance of spatial distribution of stemness and proliferation state in determining tumor radioresponse. *Math Model Nat Phenom* 2009;4:117-33.

31. Gerlee P, Anderson ARA. An evolutionary hybrid cellular automaton model of solid tumour growth. *J Theor Biol* 2007;246:583–603.
32. Mansury Y, Kimura M, Lobo J, Deisboeck TS. Emerging patterns in tumor systems: simulating the dynamics of multicellular clusters with an agent-based spatial agglomeration model. *J Theor Biol* 2002;219:343–70.
33. Mansury Y, Diggory M, Deisboeck TS. Evolutionary game theory in an agent-based brain tumor model: exploring the 'Genotype-Phenotype' link. *J Theor Biol* 2006;238:146–56.
34. Anderson ARA, Chaplain MAJ, Rejniak KA, editors. Single-cell-based models in biology and medicine. Basel: Birkhauser; 2007.
35. Maini PK, McElwain DLS, Leavesley DI. Traveling wave model to interpret a wound-healing cell migration assay for human peritoneal mesothelial cells. *Tissue Eng* 2004;10:475–82.
36. Chao DL, Eck JT, Brash DE, Maley CC, Luebeck EG. Preneoplastic lesion growth driven by the death of adjacent normal stem cells. *Proc Natl Acad Sci U S A* 2008;105:15034–9.
37. Galle J, Hoffmann M, Aust G. From single cells to tissue architecture—a bottom-up approach to modelling the spatio-temporal organisation of complex multi-cellular systems. *J Math Biol* 2009;58:261–83.
38. Brú A, Albertos S, Subizza JL, García-Asenjo JL, Brú I. The universal dynamics of tumor growth. *Biophys J* 2003;85:2948–61.
39. Brú A, Casero D. The effect of pressure on the growth of tumour cell colonies. *J Theor Biol* 2006;243:171–80.
40. Roose T, Netti PA, Munn LL, Boucher Y, Jain RK. Solid stress generated by spheroid growth estimated using a linear poroelasticity model. *Microvasc Res* 2003;66:204–12.
41. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997;33:787–91.
42. Edsjö A, Holmquist L, Pählman S. Neuroblastoma as an experimental model for neuronal differentiation and hypoxia-induced tumor cell dedifferentiation. *Semin Cancer Biol* 2007;17:248–56.
43. Tysnes BB, Bjerkvig R. Cancer initiation and progression: involvement of stem cells and the microenvironment. *Biochim Biophys Acta* 2007;1775:283–9.
44. Boman BM, Wicha MS, Fields JZ, Runquist OA. Symmetric division of cancer stem cells—a key mechanism in tumor growth that should be targeted in future therapeutic approaches. *Clin Pharmacol Ther* 2007;81:893–8.
45. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* 2006;441:1068–74.
46. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008;8:755–68.
47. Norton L. Conceptual and practical implications of breast tissue geometry: toward a more effective, less toxic therapy. *Oncologist* 2005;10:370–81.
48. Enderling H, Hlatky L, Hahnfeldt P. Migration Rules - tumours are conglomerates of self-metastases. *Br J Cancer* 2009;100:1917–25.
49. Prehn RT. The inhibition of tumor growth by tumor mass. *Cancer Res* 1991;51:2–4.
50. Ehemann V, Sykora J, Vera-Delgado J, Lange A, Otto HF. Flow cytometric detection of spontaneous apoptosis in human breast cancer using the TUNEL-technique. *Cancer Lett* 2003;194:125–31.
51. Meggiato T, Calabrese F, Valente M, Favaretto E, Baliello E, Del Favero G. Spontaneous apoptosis and proliferation in human pancreatic cancer. *Pancreas* 2000;20:117–22.
52. Mackenzie IC. Retention of stem cell patterns in malignant cell lines. *Cell Prolif* 2005;38:347–55.
53. Lynch MD. The role of cellular senescence may be to prevent proliferation of neighboring cells within stem cell niches. *Ann N Y Acad Sci* 2004;1019:191–4.
54. Diehn M, Cho RW, Lobo NA, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 2009;458:780–3.
55. Gangemi R, Paleari L, Orengo AM, et al. Cancer stem cells: a new paradigm for understanding tumor growth and progression and drug resistance. *Curr Med Chem* 2009;16:1688–703.
56. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukemia. *Nature* 2005;435:1267–70.
57. Hermann PC, Huber SL, Herrler T, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;1:313–23.
58. Isaacs JT. New strategies for the medical treatment of prostate cancer. *BJU Int* 2005;96:35–40.
59. te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP. DNA damage is able to induce senescence in tumor cells *in vitro* and *in vivo*. *Cancer Res* 2002;62:1876–83.
60. Bearer EL, Lowengrub JS, Frieboes HB, et al. Multi-parameter computational modeling of tumor invasion. *Cancer Res* 2009;69:4493–501.