Problems of somatic mutation and cancer

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Summary

Somatic mutation plays a key role in transforming normal cells into cancerous cells. The analysis of cancer progression therefore requires the study of how point mutations and chromosomal mutations accumulate in cellular lineages. The spread of somatic mutations depends on the mutation rate, the number of cell divisions in the history of a cellular lineage, and the nature of competition between different cellular lineages. We consider how various aspects of tissue architecture and cellular competition affect the pace of mutation accumulation. We also discuss the rise and fall of somatic mutation rates during cancer progression. ***BioEssays*** **26:291–299, 2004. © 2004 Wiley Periodicals, Inc.**

Introduction

It has often been noted that cancer arises from a Darwinian process of mutation and selection among somatic cells. But there is a unique aspect of cancer as an evolutionary system that has not been emphasized. Many cellular genes function primarily to repress the competitive success of their bearers. Although natural selection can sometimes favor self-restraint,[1,2] the unusual aspect of cells comes from the great number of cellular genes that enforce reproductive prudence. Such prudence arose as a necessary component of multicellularity.[1,3]

Because selection at the organismal level favors restrained cellular reproduction, many genes are tuned to keep rates of cellular reproduction far below their potential. Somatic point mutations and chromosomal mutations can therefore more easily increase cellular division or decrease cellular death than could mutations improve birth and death rates in other evolutionary systems. Put another way, a much higher proportion of mutations will be advantageous at the cellular level than at the organismal level. Somatic mutations that enhance cellular competitiveness may act directly by speeding the rate of cellular reproduction (oncogenes) or indirectly by releasing constraints on cellular reproduction (tumor suppressor genes). The great importance of mutation in cancer progression justifies special attention to the nature of somatic mutation.

We focus on the rate processes that govern the accumulation of somatic mutations. Key rate processes include the origin of somatic mutations, the accumulation of mutations in cellular lineages, and the spread of mutated cell lines in competition with other cellular lineages. Prevention of cancer largely means control of the rate at which cellular lineages accumulate mutations. Through the study of rate processes, one can begin to understand how different tissue architectures affect cancer progression and how mutation rates translate into rates of progression.

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The primacy of somatic mutation in cancer progression

Somatic mutation is not the only process that influences progression to cancer. Changes in the immune system,[4,5] hormonal status,[6,8] gene expression,[7] and signalling between tissues[9] may affect the probability and the timing of cancer progression. But somatic mutation is the only process that seems to play a key role in the progression of all tissues at all ages—it is the process, we believe, that explains most of the variation in age of cancer incidence.

Perhaps the clearest evidence for the primacy of somatic mutation comes from germline mutations[10–12] and from laboratory models with mechanisms to induce somatic mutation in particular tissues.[13] The additional mutations almost invariably shorten the progression to cancer, and often do so in ways that can be easily understood. The conclusion from this is clear: mutations have a powerful effect on the kinetics of progression. Simple calculations suggest that somatic mutation occurs often enough to be a pervasive force.[14,15]

Many other factors also influence the details of progression in particular cases. But it is a mistake to assume that the existence of such additional factors means that they must be as important as mutation for explaining the variation between individuals in the quantitative timing of progression. If one is interested in the details of why a particular tumor forms in a particular place at a particular time, then those details dominate. We are interested in the most important factors that affect the overall variation in the kinetics of progression in all

tissues. The evidence strongly supports focus on mutation as one of the key factors—perhaps the dominant factor in setting rate-limiting steps in progression.

How does the architecture of renewing epithelial tissues affect the accumulation of mutations?

Epidermal and intestinal tissue are composed of many small compartments, each compartment with no more than a few hundred to a few thousand cells.[16–20] The cells in different compartments divide independently and renew lost surface cells, with little mixing of cells between compartments. Tissue renewal occurs continuously. For example, the human intestine replaces its surface cells every few days.

Cairns[21] suggested that renewing tissues may reduce the risk of cancer by separating into long-lived stem cells and short-lived transit cells. Stem cells divide repeatedly and remain at the base of the epithelial compartments. Normally, each stem cell division gives rise to one stem cell that remains at the basal layer and one transit cell. The transit cell divides a limited number of times, producing cells that move up from the basal layer and eventually slough off from the surface. For example, recent studies of human epidermal tissue suggest that the skin renews from relatively slowly dividing basal stem cells that give rise to rapidly dividing transit lineages, each transit lineage undergoing three to five rounds of replication before sloughing from the surface.[19] Studies of gastrointestinal compartments estimate four to six rounds of division by transit lineages.[22]

The stem lineage renews the compartment and survives over time. Thus, accumulation of somatic mutations occurs mainly in the stem lineage. Mutations in transit cells or differentiated cells may sometimes contribute to tumorigenesis but, in this paper, we focus on what we believe to be the dominant rate-limiting steps, which probably occur most often in stem lineages.

Little is known about the history of stem lineages. For example, how many actively dividing stem cells renew a compartment? This remains controversial. Potten's group estimated 4–6 active stem cells in each mouse intestinal compartment;[20] other estimates range from one stem cell to more than half of all cells in a compartment.

With regard to the accumulation of mutations, a more important issue concerns the lineage history of the active stem cells. Occasionally, a stem cell may die. The dead cell may be replaced by the daughter of an active stem cell,[20] in which case the total number of divisions in the history of stem lineages continues to increase over time. Occasional loss of stem cells and replacement by other stem lineages means that, over time, each compartment is dominated by a single stem lineage even if there is more than one actively dividing stem cell at any time. Empirical studies support the idea that compartments are essentially monoclonal.[22–25]

If the same stem lineages continue to divide with increasing age, then epithelial stem lineages may divide many times. At age 60, an individual has lived about 22,000 days. Let us conservatively estimate human intestine renewal as every 7 days. Thus, people at age 60 have renewed their intestinal epithelium over 3,000 times. If we measure the age of cells as the number of divisions in their somatic history, then some stem cells in epithelial compartments may have divided 3,000 times by age 60.

If we suppose that the mutation rate per gene per cell division is about 10^{-7}, and there are about 10^5 genes, then the mutation rate per cell division in the coding region of the genome is about 10^{-2}. The average stem lineage after 3,000 divisions would have experienced about 30 mutational events. There are roughly 10^7 compartments in the colon and many also in the skin. With so many stem lineages, a large number of those lineages would experience hundreds of mutations by the later stages of life.

These calculations suggest that some other process likely controls the accumulation of mutations. Cairns[21,26] argued that stem cells may have reduced mutation rates compared with other somatic cells. Two processes may reduce mutation rates. First, in each asymmetric stem cell division, the stem lineage may retain the original DNA templates, with all new DNA copies segregating to the transit lineage. If most mutations occur in the production of new DNA strands, then most mutations would segregate to the transit lineage, and the stem lineage would accumulate fewer mutations per cell division.[27,28] Second, stem cells may be particularly prone to apoptosis in response to DNA damage, killing themselves rather than risking repair of damage.[22,29]

If these processes reduce stem cell mutation rates, then carcinogens or other accidents that kill stem cells may have a large effect on the accumulation of mutations in compartments.[26] In particular, lost stem cells must be replaced by normal, symmetric cell division with typical mutation rates that may be much higher than stem cell mutation rates. Thus, mutations may accumulate during periods in which stem cells are being regenerated.

Is there a hierarchy of stem cells to flush somatic mutations?

The problem with a separation between stem cells and transit cells comes from the accumulation of mutations in long stem lineages. A hierarchy of stem cells could reduce the accumulation of mutations.[30] We discuss two different mechanisms, which we label stochastic flushing and deterministic flushing.

In the stochastic model, each compartment retains a pool of nearly quiescent proto-stem cells. The active stem cell divides and renews the tissue for a while, but eventually dies, perhaps by apoptosis in response to DNA damage. When the stem cell dies, it is replaced by one of the cells in the quiescent pool.

If time to death of stem cells follows the typical negative exponential distribution, then across all compartments at any point in time, the age distribution of active stem lineages will approximately follow the negative exponential distribution. This distribution should not change much as the organism ages as long as a pool of proto-stem cells remains in each compartment. Long lineages would be rare and mutations would accumulate much more slowly than with only a single long stem lineage.

In the deterministic model, the compartment is divided into a series of stages. The ultimate stem lineage lives at the base and divides very rarely. Each division of the ultimate lineage flushes the stages above and keeps low the rate of mutational accumulation. At the next stage up, the secondary stem lineage divides and renews the tissue for a while, until the ultimate stem lineage below divides and replaces the secondary stem lineage. There could be more layers, but two stem layers would be enough to significantly reduce the length of stem lineages and the accumulation of mutations. Under this model, all compartments in a tissue would have approximately the same stem lineage length at any time. The average length of cellular lineages in compartments would increase steadily with age.

The problem of long lineages and mutation accumulation also arises in spermiogenesis. Perhaps some sort of stem hierarchy helps to flush germline mutations as males age. Further refinements of recent genetical techniques may allow estimation of lineage age[25] and testing of these hypotheses.

How does tissue compartment size affect the accumulation of mutations?

Little is known about the biology of stem lineages at the base of compartments. It may be that a small pool of basal stem cells divides and renews the tissue.[22,29] If so, then the accumulation of mutations in a compartment depends on the competition between cellular lineages in the stem pool. A deleterious mutation arising in one stem cell will probably cause the other stem lineages to outcompete the damaged line, deleting the mutation and preventing accumulation. An advantageous mutation that increases cellular proliferation will probably cause that aggressive cell line to outcompete its neighbors and take over the compartment. Here, ''deleterious'' and ''advantageous'' have to do with the success of cellular lineages in competition with neighbors and not with the success of the organism.

What sort of cancer-promoting mutations will be deleterious? Mutator mutations that increase chromosomal aberrations or reduce DNA repair often occur in cancer cells. Such mutators raise the mutation rate, which may promote new mutations that help to transform cell lines and raise the competitiveness of those lineages. However, at first, most new mutations caused by mutators are likely to be deleterious, and so a new mutator that causes a very high mutation rate is at risk of being lost by competition from neighbors. New mutations may be deleterious for the obvious reason that they disrupt cellular function. In addition, the apoptotic machinery of cells responds to several kinds of mutation and DNA damage, acting as an intracellular immune system against cancer that has the consequence of making many mutations lethal to the cell.

What sort of cancer-promoting mutations will be advantageous? Many classic cancer mutations to tumor suppressor genes or oncogenes increase the net rate of cellular reproduction. These mutations work by increasing the rate of progression through the cell cycle and by reducing cell death. Such mutations are advantageous to cell lineages in competition with neighbors.

The size of the stem pool affects the spread of deleterious and advantageous mutations.[31,32] In a large stem pool, oncogene and tumor suppressor mutations with increased rates of proliferation almost always succeed, whereas mutator mutations with decreased rates of proliferation rarely succeed. Put another way, natural selection among cell lineages deterministically takes its course in a large population. In small stem pools, chance events can influence which cell lineages succeed or fail. Thus, small pools increase the probability that deleterious mutator mutations spread and decrease the probability that advantageous mutations spread.

In terms of cancer risk, compartments with large stem pools may often lead to cancer progression via initial tumor suppressor and oncogene mutations and rapid cellular proliferation. By contrast, small stem pools may often begin cancer progression with mutator mutations and genetic instability.

Different tissues may vary in the size of their cellular compartments and stem pools. This stochastic model for the spread of cellular lineages in compartments predicts that tissues with smaller stem pools more often begin cancer progression via genetic instability than do tissues with larger stem pools. Renewing epithelial tissues such as skin and colon have small compartments and are therefore good candidates for early genetic instability. In general, it will be important to study the detailed structure of compartments and the architecture of tissues to understand mutation accumulation and progression to cancer Figure 1.

How do phases of initial tissue development and subsequent tissue renewal differ with regard to the accumulation of mutations?

Renewing tissues typically have two distinct phases in the history of their cellular lineages. Early in life, cellular lineages expand exponentially to form the tissue. For the remainder of life, stem cells renew the tissue by dividing to form a nearly linear cellular history. Figure 2 shows a schematic diagram of the exponential and linear phases of cellular division.

Mutations accumulate differently in the exponential and linear phases of cellular division.[33] During development,

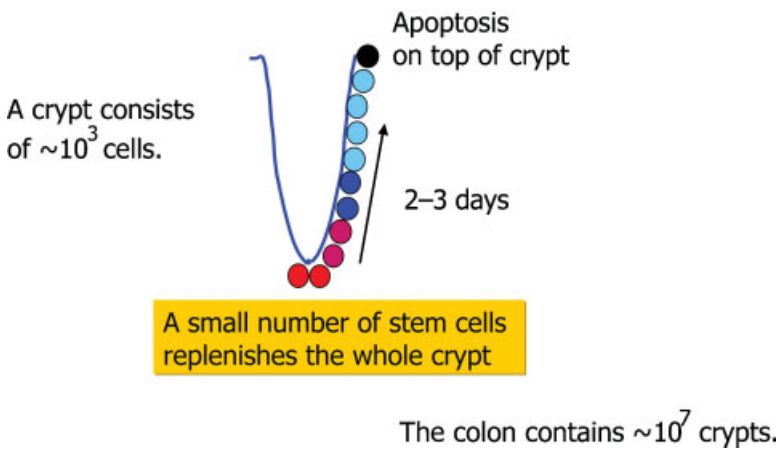

Figure 1. The structure of epithelial tissue compartments influences the accumulation of somatic mutations and progression to cancer. This figure illustrates a crypt, the compartmental unit of colon tissue. The stem cells reside at the base of the crypt. Each stem cell division typically gives rise to one stem cell that remains at the crypt base and one transit cell that moves up. The transit cell then divides several times, pushing the cells above toward the colon surface, where the surface cells undergo apoptosis and are shed. The stem cells form the only long-lived cell lineages, from which other crypt cells derive. Thus, cancer progression mostly follows the accumulation of mutations to stem cell lineages.

suppose that one initial cell divides exponentially to produce N stem cells that seed a renewing tissue such as the skin or colon. The classical Luria-Delbrück distribution[34,35] describes the probability that a frequency x of those initial stem cells carries a mutation or, equivalently, that a total of Nx initial stem cells carry mutations.

A mutation in the early rounds of exponential cell growth carries forward to all descendants, causing a high frequency of mutated cells. For this reason, the frequency of mutated cells can occasionally be very high, causing rare individuals to carry the same mutation in a large fraction of initial stem cells. By contrast, mutations during the linear history of stem cell division remain localized in a single compartment, unless cancer causes invasive growth.

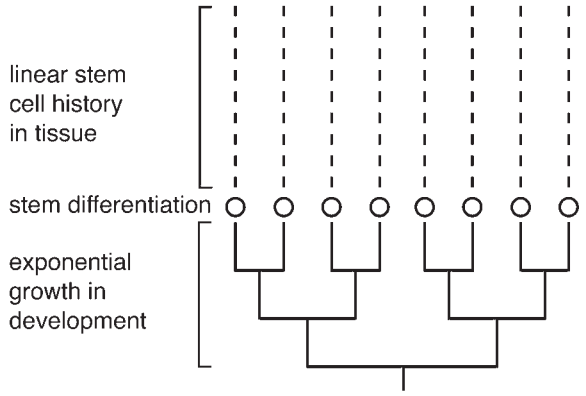

Figure 2. The phases of cellular growth in epithelial tissues. Cell populations expand exponentially during development, shown by a branching phase of division. At the end of development, stem cells differentiate in each tissue compartment. Stem cells renew each compartment by dividing to form a nearly linear cellular history—each stem cell division gives rise to one daughter stem cell that continues to renew the tissue and one daughter transit cell that divides rapidly to produce a short-lived transit lineage that fills the tissue.

The number of rounds of cellular division to produce N stem cells from one initial cell is approximately $\ln(N)$, ignoring cell death. To make $N = 10^7$ stem cells requires cellular lineages with, on average, $\ln(N) \approx 16$ cell divisions back to the initial progenitor cell; for $N = 10^9$, there are approximately $\ln(N) \approx 21$ cell divisions per lineage.

What is the probability that a stem cell carries a mutation at the end of exponential growth and before the linear phase of division and tissue renewal? If the mutation rate per cell division during exponential growth is u_e, then the probability of mutation is roughly the mutation rate multiplied by the number of cell divisions, $\bar{x} \approx u_e \ln(N)$. The probability that any particular initial stem cell has a mutation is small, but the average number of initial stem cells with mutations, $N\bar{x}$, can be significant.

Put another way, we can expect roughly $N\bar{x}$ compartments to begin life with mutated stem cells. Those mutated compartments begin one step further along in the progression to cancer than compartments that begin with pristine stem cells. Although initially mutated compartments are only a small fraction of the total compartments, those compartments with initial mutations may contribute significantly to cancers later in life because of their much higher risk of transformation.[33]

Do mutation rates rise and then fall during transformation to cancerous growth?

Cancer progression requires broad changes in cellular physiology and often demands rapid adaptation to novel environments. This demand for change suggests that the

mutation rate may often be elevated in cancerous lineages for at least part of the time during progression.[36,37] However, the role of elevated mutation rates remains controversial. An early mutation could cause an expanding clone of stem cells, which would increase the opportunities for a second mutation to arise within a cellular lineage without the need to invoke an elevated mutation rate.[38–40] Alternatively, selection may favor bypassing slow DNA repair mechanisms in mutagenic environments to speed progress through the cell cycle, thus selection for rapid cellular proliferation may indirectly raise the mutation rate.[41,42]

The available data suggest either high point mutation rates or high rates of chromosomal aberrations or both during some stage of cancer progression. Cairns[43] noted that cancer cells may sometimes have many point mutations but do not show a raised point mutation rate when cultured in vitro. Cultured cells do often show high levels of chromosomal instability.[44] Strauss[45] found that cancer cells have a large excess of silent DNA substitutions in the *p53* gene, suggesting an excess accumulation of point mutations. It is not yet clear whether *p53* is unusual in its accumulation of mutations. However, taken together, these observations do suggest a raised rate of point mutations or chromosomal instability for at least some period during cancer progression.

High mutation rates speed adaptation in novel environments by providing greater variability, the engine of evolutionary change. But a high mutation rate also imposes a potentially large cost in a relatively stable environment, because most new mutations are deleterious. Put another way, in a stable environment, a well adapted cell line with a low to intermediate mutation rate will probably outcompete a cell line with a very high mutation rate. (See the extensive literature on bacterial mutation rates.[46–48]) Loeb[49] suggested that mutation rates may rise and then fall during cancer progression. In periods that demand rapid change and adaptation, higher mutation rates will be favored. In more stable periods, lower mutation rates will be favored.

In evolutionary theory, there is an important concept called the error threshold.[50–53] If mutation rates exceed the error threshold, then the decay in fitness from accumulated mutations outweighs any potential fitness improvements from natural selection. Eventually, the lineage suffers a mutational meltdown and goes extinct. In novel environments, rates of adaptation may be highest near the error threshold—perhaps even a temporary excursion above the error threshold may speed adaptation. But a lineage cannot remain long beyond the threshold. We suggest that rapidly progressing cancers make excursions across the error threshold, with natural selection favoring higher and then lower mutation rates as environmental novelty and adaptation to those novel environments follow.

The great benefit of very high mutation rates may be an unusual feature of cellular competition and cancer. The special benefit arises from the need to overcome the extensive genetic network that controls cellular birth and death processes. That network normally impedes renegade cellular lineages. Successful cellular competition requires disrupting several points in the control network.

We cannot cite any conclusive evidence for our theory that progression to cancer benefits by brief excursions across the error threshold. But there are a few hints in the existing literature, and we can suggest some promising directions for future study.

Changes over time in the expression of telomerase and associated chromosomal mutations provide our first example. The telomeres at the ends of chromosomes are composed of short sequences of repeated DNA. The normal DNA polymerases usually fail to replicate the last bits of the telomeres. Telomerase is a special enzyme that adds back the unreplicated ends, maintaining chromosome length. In the absence of telomerase, the ends tend to shorten with each replication. As telomeres shorten, the uncapped ends lead to double-stranded DNA breaks. In normal cells, those breaks stop the cell cycle.[54]

Cancerous lineages may first progress through a telomerase negative phase, leading to uncapped telomere ends.[55] Mutations to the normal cell-cycle stop signal allow cells to continue proliferating with uncapped telomeres. This causes high rates of chromosomal breakage, rejoining, and loss. Rapid karyotypic change can lead to the several additional mutational steps required to develop aggressive cancer. However, the high rates of chromosomal mutations and additional decay of telomeres may limit further success of chromosomally abnormal and potentially aggressive cancer lineages.

Once a high rate of cellular proliferation and aggressive cancerous growth has been established, a cancerous lineage may require stabilization of telomeres to prevent mutational decay. This may be accomplished by expression of telomerase, which probably slows the rate of chromosomal mutation and evolution. An off-early and on-late telomerase pattern could therefore give rise to a burst of chromosomal mutation early in cancer progression, followed by partial chromosomal stabilization that causes genetic instability at a lower rate than during the peak favored early in progression, but still higher than normal. High telomerase expression may also have other cancer-stimulating effects.[56,57] If possible, it would be interesting to study the consequences for cellular fitness of later-stage reduced chromosomal instability independently of other effects on cancer progression.

Do other mutational processes also increase during early cancer development and then decline later? One possibility is that the rate of change to methylation patterns rises and then falls during cancer development. Methylation of CpG dinucleotides affects levels of gene expression.[58] Increased methylation of promoter regions lowers or silences gene expression, perhaps by changing chromatin structure. Several candidate

tumor-suppressor genes are silenced by promoter hypermethylation in certain cancers; those genes often lack DNA mutations in cancers, suggesting that altered phenotypes arise from changes in methylation.[58]

Promoter CpG hypermethylation can develop gradually and progressively, spreading from heavily methylated flanking regions toward the transcription start site of the gene.[59,60] The spreading of hypermethylation occurs heterogeneously in different cells.[59,61] Such heterogeneity may cause quantitatively varying levels of gene expression between cancer cells clonally derived from a recent ancestor.[58]

Jones and Baylin[58] suggest that methylation heterogeneity plays an important role in generating the phenotypic variance that promotes successful metastasis. For example, loss of CDH1 expression enhances metastatic characters.[59] Cellular heterogeneity in hypermethylation of the *CDH1* promoter occurs both in primary and metastatic tumors and in vitro. Selection for cell invasion in vitro favors those cells with the most densely methylated promoters and lowest expression of CDH1. Selection for growth in cell clusters, which mimics growth at distant metastatic sites, favors less densely methylated *CDH1* promoters and the re-expression of CDH1.[59] These observations emphasize how mutational mechanisms can affect the nature and the pace of evolutionary change in tumor progression. It seems likely that the rate of change in methylation can be influenced by mutations to the DNA methyltransferases that regulate the methylation process,[62,63] allowing methylation rates to rise and fall in response to selection.

Cancer cells probably continue to change and adapt over time; periods that favor mutational bursts may come and go. Mechanisms such as telomerase expression and methylation intensity exist for raising and then lowering mutation rates, beyond the usual systems of DNA repair. The expression of DNA repair systems may also be modulated over the course of cancer progression, allowing mutational bursts during strong environmental challenge and lowering of mutation during periods of relative environmental stability. All of this highlights the need for more information on the rates of different kinds of mutational processes, which will be required for a quantitative understanding of tumorigenesis.

Does horizontal gene transfer occur?

Tumor progression is probably often associated with extensive cell death that does not follow the orderly apoptotic process. The dying cells may spill significant amounts of DNA, which may then be taken up by neighboring cells. Studies of injected, naked DNA show that mammalian cells can take up, incorporate, and express extrinsic DNA in vivo.[64] We wonder whether occasional horizontal gene transfer of this sort sometimes plays a role in bringing together particularly aggressive combinations of cancer-promoting mutations. If so, the subsequent expansion of the successful recombinant cell would soon render the cancer nearly monoclonal, hiding the history of horizontal gene transfer. If the cancer has spread to different sites, it may be possible to infer the recombination by comparison of genotypes from different sites.

Conclusions

We have discussed how somatic mutations accumulate in cellular lineages. Cairns[21] introduced the subject by emphasizing three points about renewing epithelial tissue. First, the architectural division between long-lived stem lineages and short-lived transit lineages flushes compartments of somatic mutations in the transit lines. This shifts the burden of mutation accumulation to the stem lineages. Second, the division of epidermal and intestinal tissue into many small, independent compartments reduces competition between cellular lineages and protects against the spread of aggressive cell lines. Third, from these architectural considerations, Cairns[21] predicted that stem lineages have mechanisms to reduce the mutation rate per cell division.

We have extended ideas about tissue architecture and the accumulation of mutations in cell lineages to several additional topics (Table 1). After initial discussion of tissue architecture, our second topic focused on how a hierarchy of stem lineages within a compartment could reduce the accumulation of mutations. One possibility is that each compartment retains a pool of nearly quiescent proto-stem cells. The active stem cell divides and renews the tissue for a while, but eventually dies, perhaps by apoptosis in response to DNA damage. When the stem cell dies, it is replaced by one of the cells in the quiescent pool. This stochastic model predicts a negative exponential distribution for the length (number of divisions) of cellular lineages across compartments. This distribution should remain relatively stable throughout life, as long as a pool of proto-stem cells remains in each compartment.

Alternatively, the stem hierarchy could be arranged as a series of stages from the base of the compartment to the top. The ultimate stem lineage lives at the base and divides very rarely. Each division of the ultimate lineage flushes the stages above and keeps low the rate of mutational accumulation. Under this deterministic model, all compartments in a tissue would have approximately the same stem lineage length at any time. As the organisms ages, the average length of cellular lineages in compartments would increase.

Our third topic focused on the number of competing stem cells in compartments.[31] In a large stem pool, oncogene and tumor suppressor mutations that cause increased rates of proliferation almost always succeed, whereas mutators that cause very high mutation rates and decreased cellular proliferation rarely succeed. Put another way, natural selection among cell lineages deterministically takes its course in a large population. In small stem pools, chance events can influence which cell lineages succeed or fail. Thus, small pools increase

Table 1. Summary of problems and predictions

Problems	Assumptions and Predictions
1. Tissue architecture	Separation of stem and transit lineages
	Division into compartments
	P: Reduced stem mutation rates
2. Hierarchy of stem cells	Pool of quiescent proto-stem cells
	Stochastic death of active stem line
	P: Negative exponential distribution of lineage lengths across compartments, distribution relatively stable with age
	Basal stem lineage divides rarely and flushes levels above
	P: Similar lineage lengths across compartments, average lineage lengths increases with age
3. Compartment size	Mutations to oncogenes and tumor suppressor genes increase cellular proliferation
	Mutators that cause very high mutation rates decrease cellular proliferation in the short term
	Stem lineages compete in a compartmental pool
	P: Large stem pools are relatively favorable for initial oncogene and tumor suppressor gene mutations that increase cellular proliferation
	P: Small stem pools are relatively favorable for initial mutator mutations and genetic instability that decrease cellular proliferation
4. Phases of tissue growth	Cell lineages expand exponentially during development
	Renewing tissues have linear stem lineages
	P: Mutations during development seed compartments with mutated stem cells that predispose those compartments to cancer
5. Fluctuating mutation rates	During progression, chromosomal instability rises and then falls as telomeres shorten and telomerase is activated
	Mutation rates are regulated by control of DNA repair and methylation processes
	P: Mutation rates rise and fall during progression in response to environmental challenge and environmental stability
6. Horizontal gene transfer	Dying cancer cells may bypass normal apoptosis and spill DNA
	P: Naked DNA may be taken up and expressed by cancer cells, creating new cancer genotypes

Lines beginning with "P:" contain predictions.

the probability that deleterious mutator mutations spread and decrease the probability that advantageous mutations spread.

In terms of cancer risk, this model predicts that compartments with large stem pools most often begin cancer progression via initial tumor suppressor and oncogene mutations and rapid cellular proliferation. By contrast, small stem pools may often begin cancer progression with mutator mutations and genetic instability.

The fourth topic distinguished the exponential period of tissue growth during development from the linear history of stem cell divisions within compartments.[33] Mutations arising during the exponential phase seed tissues with stem cells carrying mutations that predispose to cancer. This model predicts that some of the risk of late-life epithelial cancer may be set during development. If so, then the number of predisposing mutations per individual will vary according to the Luria-Delbück distribution. Those individuals with many predisposed mutant stem cells are likely to develop multiple independent tumors relatively early in life, whereas those individuals with few predisposed stem cells are likely to develop few tumors relatively late in life. This idea could be tested with inbred rodents.

Our fifth topic considered whether mutation rates rise and then fall during cancer progression. Cancer lineages sometimes go through a phase in which shortened telomeres lead to widespread chromosomal aberrations and significant genomic rearrangements. Later stages of cancer progression often express telomerase, which stabilizes telomeres and reduces the rate of chromosomal instability. This pattern of rising and then falling chromosomal instability led us to consider the evolutionary forces on mutation rate during cancer progression.

We suggested that mutation rates may often rise and then fall during cancer progression. In periods that demand rapid adaptation, higher mutation rates will be favored. In more stable periods, lower mutation rates will be favored. We noted that periods of very high mutation may be temporary excursions across the error threshold—a threshold of mutation rate above which lineages cannot long sustain themselves in the face of mutational decay.

There are many mechanisms that can raise mutation rates. Chromosomal instability increases genomic rearrangements, defective DNA repair raises the rate of point mutations, and processes that control methylation can alter the rate of change in methylation patterns and gene regulation. It would be interesting to compare changes in these mutational mechanisms during periods when lineages face new environmental challenges and during periods of relative environmental stability.

Finally, we speculated that horizontal gene transfer may occur between cancer cells in a tumor, providing another source of genetic novelty in addition to mutation.

These different problems show that more attention to somatic mutation, cellular lineages, and tissue architecture will provide new insights into cancer dynamics.

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