Patterns of Cell Division and the Risk of Cancer

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ABSTRACT

Epidermal and intestinal tissues divide throughout life to replace lost surface cells. These renewing tissues have long-lived basal stem cell lineages that divide many times, each division producing one stem cell 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. If mutation rates are the same in stem and transit divisions, we show that minimal cancer risk is obtained by using the fewest possible stem divisions subject to the constraints imposed by the need to renew the tissue. In this case, stem cells are a necessary risk imposed by the constraints of tissue architecture. Cairns suggested that stem cells may have lower mutation rates than transit cells do. We develop a mathematical model to study the consequences of different stem and transit mutation rates. Our model shows that stem cell mutation rates two or three orders of magnitude less than transit mutation rates may favor relatively more stem divisions and fewer transit divisions, perhaps explaining how renewing tissues allocate cell divisions between long stem and short transit lineages.

CANCER follows from a series of somatic mutations. Renewing epithelial tissues such as the skin or colon may be at particular risk because of the large number of cell replications over a lifetime. CAIRNS (1975) 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 tissue. 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 (JANES *et al.* 2002). Studies of gastrointestinal crypts estimate four to six rounds of division by transit lineages (BACH *et al.* 2000).

Each piece of renewing tissue must produce a certain number of cells over a lifetime. Different patterns of stem cell and transit cell divisions can give rise to the same total number of cells. For example, stem cells may divide rarely, each division producing a transit cell that divides many times before its descendants slough from the surface. Or the stem cells may divide frequently, each division producing a transit cell that divides only a few times before sloughing.

We study a simple model for the number of stem and transit cell divisions that minimize the risk of cancer. We show that minimal cancer risk is obtained by using the fewest possible stem divisions subject to the constraints imposed by the need to renew the tissue. In this case, long-lived stem lineages are a necessary risk imposed by the constraints of tissue architecture.

CAIRNS (1975, 2002) suggested that stem cells mutate less frequently than transit cells, favoring a separation into long-lived stem lineages and short-lived transit lineages. We calculate the optimal length of stem and transit lineages for different combinations of stem and transit mutation rates. If stem mutation rates are sufficiently lower than transit mutation rates, then selection does favor a split of the cell lineages into a permanent stem line and a series of temporary transit lineages.

To initiate cancer, the transit cells may require more mutations than the stem cells. The additional mutations may, for example, cause the transit cells to stick within the tissue and avoid being sloughed from the surface. We show that if transit cells require more mutations than stem cells do, avoidance of cancer favors a shift of cell divisions into longer transit lineages and shorter stem lineages.

PRELIMINARY MODEL OF CELL DIVISION AND CANCER RISK

A renewing epithelial tissue imposes constraints on the pattern of cell division and cell death. A certain number of surface cells regularly die and slough off.

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Cell division in subsurface layers replaces the lost cells. Before considering a particular model of cell renewal, it is useful to consider how patterns of cell division affect cancer risk in the absence of constraints on which cells die and which cells divide.

In the simplest model, all cell divisions have the same mutation rate and there is no cell death. We start with a single cell. The tissue must continue to divide its cells until it has a total of k cells. In the absence of cell death, this requires $k - 1$ cell divisions. Cancer arises if at least one cell acquires m mutations. We assume that all mutations act dominantly in the manner of oncogenes. This assumption of dominance simplifies the analysis. The same approach and qualitative conclusions would apply to recessive tumor suppressor loci.

What sort of topology for the history of cellular lineages minimizes the risk of cancer? One way to describe topology is the evenness in the length of cellular lineages. For example, each division could give rise to one daughter that does not divide again and one parent that continues division. To make $k = 2^n$ cells would require a parental stem lineage with a length of $2^n - 1$ cell divisions. Alternatively, each cell division could give rise to two daughter cells, each of which divides. The resulting binary tree would produce 2^n final cells, each cell ending a cellular lineage with n divisions in its history.

If $m = 1$, then topology does not matter because all topologies have $k - 1$ cell divisions, and the risk of one mutation depends only on the number of cell divisions.

If $m > 1$, then two or more mutations are needed to cause cancer. Suppose we pick any cell from the total history of cells produced in a tissue. The risk of cancer in that cell increases exponentially with the number of cell divisions, t, back to the original progenitor cell. This exponential increase occurs because the risk of cancer rises with cell divisions in proportion to t^m.

At any time in the history of cell divisions, the extant cells will have a variable number t of cell division steps back to the original progenitor cell. For the next cell division, the smallest cancer risk is achieved by using the cell with the smallest t, that is, the shortest lineage. Smallest t is best because risk increases exponentially with t. If we always add cell divisions to the shortest extant lineage, the cellular history will develop as a binary tree with all tips as close as possible to equal length from the progenitor.

Thus, in the absence of constraints imposed by particular patterns of cell death, binary cell division minimizes cancer risk. With cell death, it is best to minimize the length of long lineages.

More cell death means more cell divisions, longer cellular lineages, and greater risk of cancer. Thus, apoptosis, which can limit cancer by weeding out potentially dangerous cells, also imposes a risk because cell replacement requires increasing the length of cellular lineages.

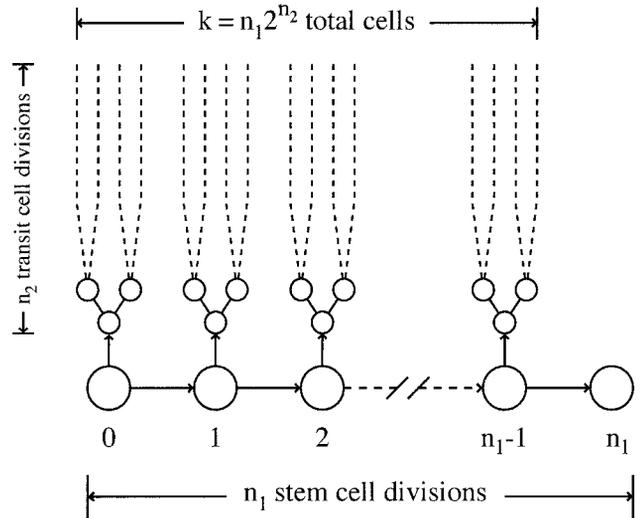Renewing tissues impose a particularly dangerous sort

FIGURE 1.—The pattern of cell division giving rise to a total of k cells. The single, initial cell divides to produce a stem cell and a transit lineage. Each transit lineage divides n_2 times, yielding 2^{n_2} cells. The stem lineage divides n_1 times, producing a total of $k = n_1 2^{n_2}$ cells.

of constraint on cellular lineages. The constant cell death forces those transit lineages terminating at the surface to be relatively short and requires the maintenance of long-lived stem lineages to renew the tissue.

RENEWING EPITHELIAL TISSUE

We assume that a single basal epithelial cell must divide to produce k cells over a lifetime. We study how the risk of cancer depends on the separation of cell divisions into the stem lineage and a series of transit lineages. In this model, m mutations to the stem lineage or $m + y$ mutations to the transit lineage cause cancer.

In our model, the m mutations must occur in m particular genes. Alternatively, one could assume a pool of $M \gg m$ mutable genes, among which only m mutations are needed to cause cancer. The larger pool of potentially cancer-inducing genes would increase the effective mutation rates, the risk of cancer, and some of the quantitative details of the models that follow. But the main qualitative features are similar under either set of assumptions about the number of mutable genes.

Figure 1 shows the pattern of cell division giving rise to k total cells. The stem lineage divides n_1 times and each transit lineage divides n_2 times, giving a total of $k = n_1 2^{n_2}$ cells. It is sometimes useful to express the parameters such that $k = 2^N = 2^{N-n_2} 2^{n_2}$ with $n_1 = 2^{N-n_2}$. Thus, the length of the stem lineage, n_1, increases exponentially as the number of transit divisions, n_2, declines.

Equal mutation rates in stem and transit cells: Suppose that stem and transit lineages have the same mutation rate. Then the result of the previous section implies that the best architecture minimizes the length of the

longest lineages because of the exponential increase in risk with lineage length. Thus, the smallest number of stem divisions and the longest possible transit lineages minimize the risk of cancer, subject to the architectural constraint for a renewing tissue that requires a certain rate of cell loss from the transit lineages. In this case, the maintenance of long-lived stem lineages arises from the architectural constraints of the tissue rather than from a scheme of cell division that gives the lowest possible risk of cancer. With equal stem and transit mutation rates, long-lived stem lineages are a necessary risk imposed by tissue architecture.

Different mutation rates in stem and transit cells: CAIRNS (1975, 2002) suggested that stem cells may have reduced mutation rates compared with transit lineages. This would favor more divisions in the stem lineage and fewer divisions per transit lineage.

Cairns argued that, in each cell division, the stem lineage retains the original DNA templates, and all new DNA copies segregate 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. Cairns cites some evidence in favor of stem cells retaining templates, suggesting that more empirical studies on this topic would be valuable.

Different levels of exposure to mutagens may also cause different mutation rates in stem and transit lineages. In the skin and intestine, the stem cells reside several layers below the surface. By contrast, the transit lineages occur at the upper tissue layers. The upper transit cells may be exposed to numerous mutagens, whereas the deeper stem cells may be partially protected. Alternatively, the stem cells may divide more slowly than the transit cells, allowing more time in the stem lineage for DNA damage checkpoints and repair. Or, stem cells may be particularly prone to apoptosis in response to DNA damage, killing themselves rather than risking the repair of damage (CAIRNS 2002).

We assume that a stem cell produces one daughter stem cell that inherits mutated genes with probability u_s per gene and one daughter transit cell that inherits mutated genes with probability u_t per gene. Further divisions by transit cells have a mutation rate of u_t.

We calculate the probability of developing cancer for two mutations, $m = 2$ and $y = 0$. Consider first the probability of two mutations to a cell in a transit lineage with n_2 cell divisions, assuming that the initial cell has no mutations,

$$T_2(n_2) \approx \sum_{i=1}^{n_2} 2^i (u_t^2 + 2u_t T_1(n_2 - i)),$$

where there are 2^i cells after the ith round of cell division, each cell with a chance u_t^2 of getting two mutations and a chance $2u_t$ of getting one mutation. If a cell suffers one mutation, the risk of at least one additional mutation in the descendant lineage is $T_1(n_2 - i) = 1 -$

$e^{-u_t d}$, where $d = 2(2^{n_2-i} - 1)$ is the number of branches in the descendant cell lineage along which mutations can occur.

The total risk of two mutations and cancer accumulates along the stem lineage as

$$R_2(n_1, n_2) \approx \sum_{i=1}^{n_1} e^{-2u_s(i-1)}[2u_s R_1(n_1 - i + 1, n_2)$$
$$+ (1 - 2u_s) T_2(n_2)],$$

where the probability at step i that a first mutation in the stem lineage has not occurred is $e^{-2u_s(i-1)}$, the probability of the first mutation at the ith step is $2u_s$, the term

$$R_1(n_1 - i + 1, n_2) = 1 - e^{-(n_1-i+1)(u_s + u_t(2^{n_2+1}-1))}$$

is the risk of at least one mutation in the descendant branches in the cellular lineage including the current cell, and, finally, no mutations occur in the ith stem cell with probability $1 - 2u_s$ and two mutations occur in its descendant transit lineage with probability $T_2(n_2)$. Using the geometric series, the above approximation for R_2 can be written equivalently as

$$R_2 \approx [2u_s + (1 - 2u_s) T_2(n_2)] \frac{1 - e^{-2u_s n_1}}{1 - e^{-2u_s}}$$
$$- 2u_s e^{-n_1(z+2u_s)} \frac{e^{n_1 z} - 1}{e^z - 1}, \tag{1}$$

where $z = u_t(2^{n_2+1} - 1) - u_s$.

We tested the quality of the approximation in Equation 1 by running replicates of a Monte Carlo simulation of cellular lineages with mutation. Figure 2a shows a match between the shape of the curves from the mathematical approximation and from the computer simulation.

Figure 3 shows the risk of at least one cell carrying two mutations and causing cancer. The plots show risk for different combinations of stem and transit lengths, with the x-axis giving the transit length n_2 corresponding with a stem length of $n_1 = 2^{N-n_2}$. In all cases, $u_t = 10^{-6}$. Each plot shows different values of u_s. The three curves in each plot from bottom to top illustrate the risk for $N = 20, 25$, and 30, which corresponds to the production of 10^6–10^9 final transit cells from a single original stem cell.

Note that the choice of n_2 that minimizes risk changes little with the need to produce more cells. Instead, lowest risk occurs by elongating the stem lineage, keeping a constant number of transit divisions. Lower stem cell mutation rates favor a shift to more stem and fewer transit divisions, as expected.

The risk of three mutations ($m = 3$, $y = 0$) is

$$R_3(n_1, n_2) \approx \sum_{i=1}^{n_1} e^{-3u_s(i-1)}(3u_s R_2(n_1 - i + 1, n_2)$$
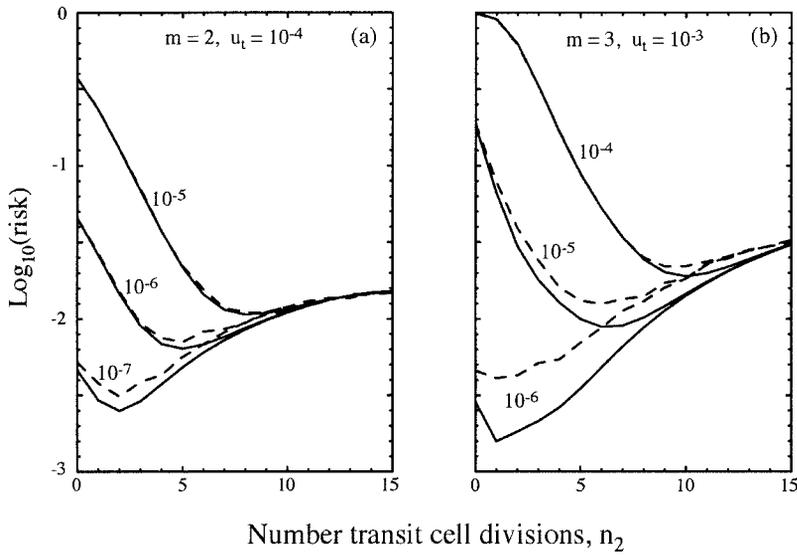$$+ (1 - 3u_s) T_3(n_2)) \tag{2}$$

FIGURE 2.—Comparisons of theoretical approximations and Monte Carlo simulations for the risk of cancer. (a) Two mutations cause cancer, $m = 2$, and the total number of cells made is $k = 2^{15}$. The transit mutation rate is $u_t = 10^{-4}$, and the stem mutation rate varies from 10^{-7} to 10^{-5} as shown above each curve. The solid line shows the theoretical approximation from Equation 1. The dashed curve shows the outcome from repeated trials of a Monte Carlo simulation. (b) Two mutations cause cancer, $m = 3$. Other parameters are as in a or as labeled on the plot. The theoretical approximation is from Equation 2. Note that the approximation shows more divergence from the simulations as u_s falls toward u_t^2, that is, as the frequency of stem mutations per cell division falls toward the frequency of two simultaneous transit mutations per cell division. Thus, the approximations work best when $u_s \gg u_t^2$. We used higher mutation rates in these plots than in later examples to obtain a sufficient number of cellular histories with cancer.

with the probability of three hits in a transit lineage of

$$T_3(n_2) \approx \sum_{i=1}^{n_2} 2^i (u_t^3 + 3u_t^2 T_1(n_2 - i) + 3u_t T_2(n_2 - i)).$$

Figure 4 shows the risk of at least one cell carrying three mutations. Compared with two mutations to disease, the need for three mutations favors a shift to shorter stem lineages and longer transit lineages. This shift occurs because risk in a particular cellular lineage rises with the third power of the number of cell divisions in that lineage, putting a higher cost on a long stem lineage than on that with two mutations. Figure 2b tests

the theoretical approximation against results from Monte Carlo simulations.

Transit cells require more mutations to become cancerous: Transit cells move toward the surface and slough because of pressure from below by continually dividing cells. Thus, transit cells may require mutations to avoid sloughing to cause cancer. For example, an additional mutation that makes a transit cell surface sticky may prevent it from shedding.

Suppose that transit cells require more mutations to cause cancer than stem cells do. As more transit mutations are needed to cause cancer, the risk of cancer is
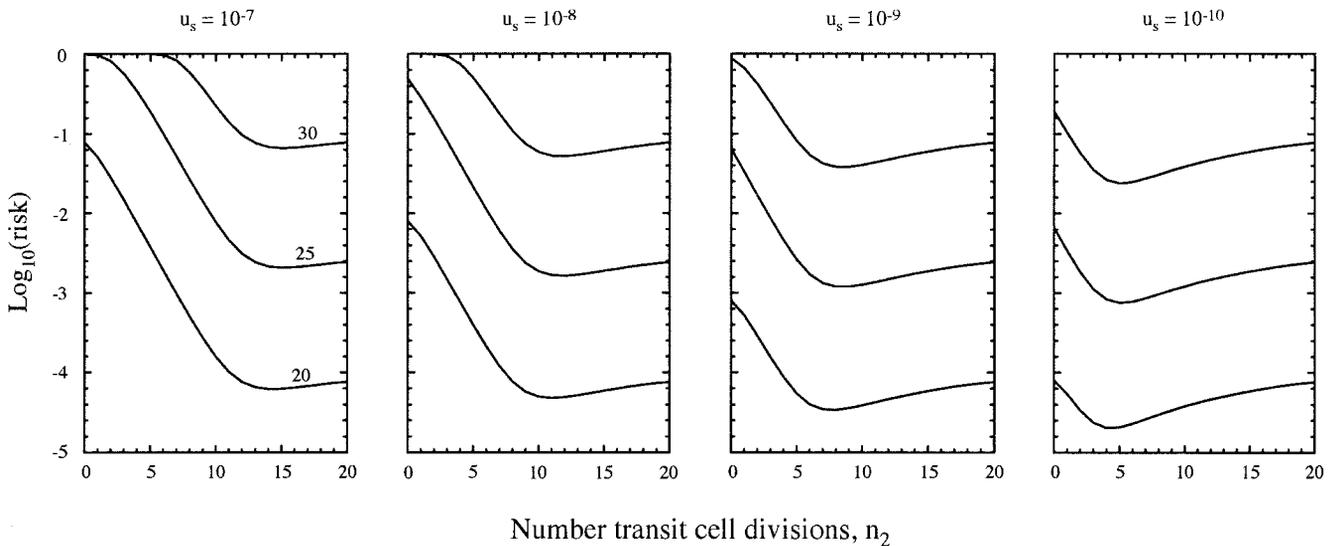

FIGURE 3.—Risk of a cell carrying two mutations, leading to cancer. The x-axis gives the transit length n_2 corresponding with a stem length of $n_1 = 2^{N-n_2}$. Each plot shows different values of u_s, with $u_t = 10^{-6}$ in all cases. The three curves in each plot from bottom to top illustrate the risk for $N = 20$, 25, and 30; calculated from Equation 1.
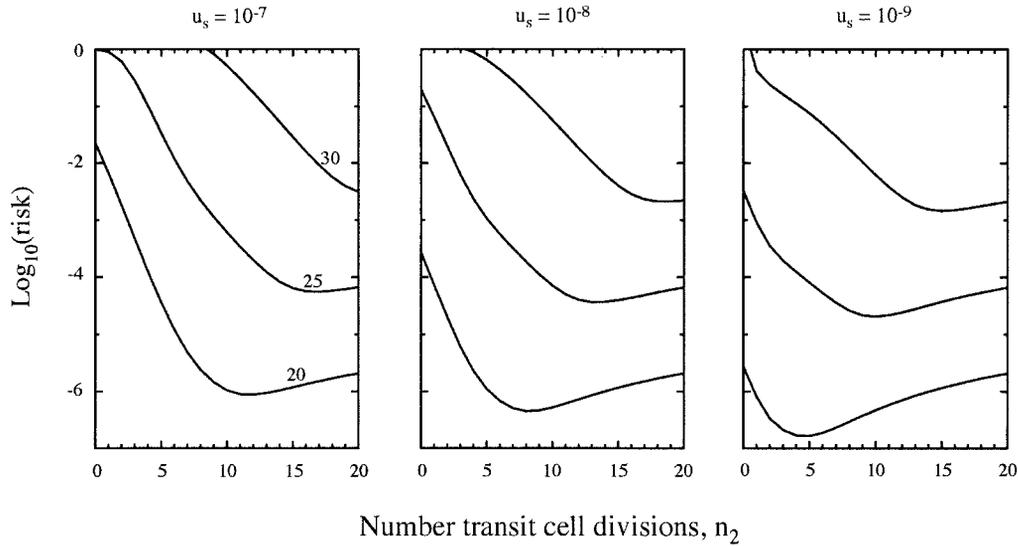
FIGURE 4.—Risk of a cell carrying three mutations, leading to cancer. The x-axis gives the transit length n_2 corresponding with a stem length of $n_1 = 2^{N-n_2}$. Each plot shows different values of u_s, with $u_t = 10^{-5}$ in all cases. The three curves in each panel from bottom to top illustrate the risk for $N = 20$, 25, and 30; calculated from Equation 2.

reduced by shifting more cell divisions away from the stem lineage and into transit lineages. This shift is favored because the extra transit mutations to cause disease protect the transit lineages and reduce their risk relative to stem cells.

DISCUSSION

Three factors have been proposed to favor a split into a permanent stem lineage and a series of short transit lineages. First, CAIRNS (1975) argued that transit lineages discard their mutations; thus it would be advantageous to shift cell divisions to those disposable lineages. We showed that if mutation rates are the same in stem and transit lineages, then minimal risk occurs with the shortest stem lineage required to satisfy the constraints imposed by the architecture of a renewing tissue. A renewing tissue must continually produce new cells to replace the specially differentiated and short-lived surface layers.

Second, transit cells may require more mutations to cause cancer than stem cells do because transit cells would normally be discarded after a short time. Disposable mutations and extra protection in the transit lineage favor a shift to longer transit lineages and shorter stem lineages. Thus, neither disposable transit mutations nor lower risk in transit cells can explain the maintenance of the stem-transit division as a mechanism to reduce cancer risk.

Third, CAIRNS (1975, 2002) suggested that stem lineages have lower mutation rates than transit lineages. Risk would thus be reduced by more lower-risk stem divisions and fewer higher-risk transit divisions. Several factors may reduce the relative mutation rate of stem lineages: stem cells may keep the DNA templates and segregate the new, error-prone copies to daughter transit lineages; stem cells may induce apoptosis rather than error-prone repair in response to DNA damage; stem

cells may divide more slowly, allowing more time for accurate replication; and stem cells may experience lower carcinogen doses than the surface-exposed transit cells do. These factors could plausibly reduce stem mutation rates relative to transit cell mutation rates. Cairns cites some evidence in favor of these factors. Firm conclusions require further studies.

We studied the quantitative effects of different mutation rates in stem and transit lineages. Figure 3 shows the risk of two mutations arising in at least one cell given a particular separation into stem and transit lineages. Between 5 and 15 transit divisions minimize cancer risk. The lower end of this range matches the 3 to 5 transit divisions of the skin (JANES *et al.* 2002) and 4 to 6 transit divisions of the intestine (BACH *et al.* 2000). In Figure 3, the mutation rate per gene in transit divisions is 10^{-6} and the stem mutation rate varies from 10^{-7} to 10^{-10}. Figure 4 shows similar plots for the risk of three mutations arising in at least one cell.

We have focused on a small number of mutations for transformation to cancer. It would be more accurate to emphasize that many mutations are probably required to transform a lineage, but that only a small number of mutations are rate limiting for transformation (KNUDSON 1993; HANAHAN and WEINBERG 2000). For example, the rate-limiting steps may have to do with reduced DNA repair or disabling of apoptotic mechanisms that kill cells with DNA damage. Once past these steps, other mutational steps that are not rate limiting may accumulate rapidly.

Epithelial cancers in humans probably require more than two or three rate-limiting mutations (KNUDSON 1993; HANAHAN and WEINBERG 2000). However, an individual may have in early life one or more mutations in several epithelial stem cells. Suppose, for example, that a tissue is divided into $10^7 \approx 2^{23}$ compartments, each compartment forming a unit with basal stem cells and rising transit lineages that slough from the epithelial surface.

If each compartment had only one stem cell derived from a single progenitor cell, it would require >23 rounds of binary cell division to populate the compartments.

If the progenitor came from the initial $100 \approx 2^7$ cells of the early embryo, then the stem cells of the 10^7 compartments have been through at least 30 rounds of cell division. Many compartments would begin with a stem cell with one rate-limiting mutation, and some individuals would have compartments that begin life with two rate-limiting mutations in disease-causing genes. Thus, an additional two or three mutations during epithelial renewal may pose some risk.

Minimizing cancer risk may not be the most important force shaping tissue architecture. Renewing tissue requires constant production of short-lived cellular lineages. This structural constraint alone could give rise to cellular histories with a combination of long-lived and short-lived lineages.

Most cancers occur in older individuals, in which the force of natural selection is weak. Thus, at first glance, it may seem that cancer must be a relatively weak selective force. However, natural selection favors additional protections against cancer up to the point at which one more protective character would be selectively neutral (Nunney 1999). The fact that cancer is presently a weak selective force may be the consequence of how selection has in the past shaped tissue architecture and the multiple regulatory controls on DNA damage, cell proliferation, and cell death. For example, the retina has relatively small numbers of cell divisions and therefore a low risk of accumulating mutations, and it has relatively few rate-limiting regulatory controls preventing transformation to cancer. By contrast, epithelial tissues have relatively many cell divisions, a greater risk of accumulating mutations, and relatively more rate-limiting regulatory controls preventing transformation to cancer (Knudson 1993).

Cancer has probably been a very strong selective force in the evolutionary history of multicellularity. Humans have hundreds or perhaps thousands of genes to prevent cancer from occurring early in life (Vogelstein and Kinzler 2002). In mice without p53, tumors developed in >75% of the animals by 6 months of age (Malkin 2002). It may be that because cancer is such a strong selective force, it does not appear early in humans or other animals.

How much of tissue architecture is shaped to avoid diseases caused by the accumulation of somatic mutations? Our first model demonstrates that ideas such as disposable transit lineages cannot by themselves explain the stem-transit structure. We then calculated the risks under different stem and transit mutation rates. If stem mutation rates are lower than transit rates, then our models make two predictions. First, as the number of mutations required to cause disease rises, minimizing risk favors more transit and fewer stem divisions. Second, as the stem mutation rates decrease relative to transit rates, minimizing risk favors more stem and fewer transit divisions. It would be interesting to compare epithelial tissue architectures in organisms with different life spans, rates of tissue renewal, and relative rates of stem and transit mutations.

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