review article

Mutation selection and the natural history of cancer

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Survival of the rapidly renewing tissues of long-lived animals like man requires that they be protected against the natural selection of fitter variant cells (that is, the spontaneous appearance of cancer). This article discusses three possible protective mechanisms and shows how they could explain various features of the natural history of certain common cancers of man.

We are accustomed to thinking of the combination of natural variation and natural selection as a force for the good, that creates and maintains the fittest in a species and discards the unfit. This is the fundamental theorem of biology. But when we turn from the competition between the individuals of a species to the competition between the individual cells within a single animal, we see that natural selection has now become a liability. The dangerous mutations are now those that confer on a cell an increased survival advantage. We may therefore expect to find, especially in animals which undergo continual cell multiplication during their adult life, the evolution of mechanisms that protect the animal from being taken over by any "fitter" cells arising spontaneously during its lifetime—that is mechanisms for minimising the rate of production of variant cells and for preventing free competition between cells.

The cells of the surface epithelia that separate a typical mammal from its environment undergo continual turnover, and this turnover accounts for most of the cell division that occurs in the life of the animal. For example, a newborn rat contains some 3×10^9 cells and grows to a final value of about 6×10^{10} cells in old age; excluding any programmed cell death, the rat therefore represents a total of 6×10^{10} cell divisions. During its entire lifetime, however, the rat produces and discards about 10^{13} epithelial cells from its small intestine alone, and almost as many cells from various other epithelia[1]. Its risk of being overwhelmed by variants is, in principle at least, related to this total of 10^{13}. For man, who lives ten times as long and is one hundred times as large, there must be some 10^{16} cell divisions, and this number must somehow be reconciled with a spontaneous mutation rate of about 10^{-6} per gene per cell division[2–4]. Because most of the cell division is occurring in epithelia, that is where we may expect to find the protective mechanisms most highly developed.

Our interest in these protective mechanisms arises not least of all from the fact that they will determine how "fitter" variants are created by mutagenic agents and how such variants can be helped to supplant their normal neighbours (the process of carcinogenesis). Here we come to an added reason for concentrating on epithelia. Although most experimental cancer research, especially that using oncogenic viruses, deals with tumours of supporting tissues and blood-forming cells (the sarcomas and leukaemias), human cancer is predominately a disease of the various surface and glandular epithelia (Table 1).

This paper describes various ways in which the programme of cell renewal in epithelia may have evolved to minimise the risk from somatic mutation, and finally how certain features of natural carcinogenesis could be explained in terms of such a programme.

Lineages and the place of stem cells in programmes of cell renewal

The turnover that occurs in the self-renewing epithelia is the result of continual shedding of superficial cells balanced by continual multiplication of the deeper cells. In the simplest examples like skin, cell division is restricted to the deepest (basal) layer of cells. To keep the number of basal cells constant, one of the two daughter cells resulting from each cell division must on average remain in the basal layer and the other must escape and be discarded. Obviously there are several possible programmes of cell division that could produce this result: a regular programme of asymmetric division, where one of each pair of daughter cells is invariably exported (Fig. 1*a*); or a random choice of cells for export, the rule being simply that the available space for basal cells should be kept fully occupied; or lastly some more complex programme in which cells destined for export are allowed to undergo a certain number of divisions before they are exported from the basal layer (Fig. 1*b*). Some elegant experiments on the fate of pairs of daughter cells, arising in the rat oesophagus, suggested that the choice was random[6]. It now seems more likely, however, at least in skin[7,8] and small intestine[9], that a fairly rigid programme is operating of the kind shown in Fig. 1(*b*)—that is, most of the dividing cells produce descendants that are all ultimately exported.

An arrangement of this kind will decrease the rate of accumulation of spontaneous mutations and the risk from exposure to mutagens. The only mutations that accumulate during the life of an animal are of course those that arise in cells that themselves survive or contribute descendants that survive for the animal's lifetime—that is, the mutations must occur in the "immortal" stem cells (drawn as squares in Fig. 1). Any muta-

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tion that arises in a cell destined to be discarded will be lost (unless of course its effect is expressed quickly and serves to prevent the cell from being discarded).

The smaller the proportion of cells that are immortal stem cells, the lower the risk. In the different self-renewing systems of mammals the proportion probably ranges from one in ten (mouse skin)[8] to one in several thousand (bone marrow)[10]. But in most instances the number of immortal stem cells is not known. Note, however, that it may be much less than the number of cells capable of being promoted to immortality by force of circumstances (for example, that are capable of recolonising an epithelium or bone marrow depleted by X irradiation) or the number of cells that are still undifferentiated (the usual, but in this context less useful, meaning of the term "stem cell").

Stem cell division and the segregation of old and new DNA strands

Although the division of each immortal stem cell can produce two apparently indistinguishable daughter cells the fate of which may, in many systems, be determined simply by their position in space, it does not follow that the two daughters must be genetically equivalent and must therefore be equallv likely to contain mutations.

Most spontaneous mutations probably arise as errors during DNA replication. Because DNA is duplicated semi-conservatively, these mutations are present initially in "heterozygote" molecules with one mutant strand and one normal strand. Unless such a heterozygote molecule is converted to homozygosity by one of the DNA repair mechanisms of the cell it will, on subsequent duplication, yield one normal daughter molecule and one molecule which is mutant in both strands (Fig. 2*a*). We see at once that stem cells would be protected against errors of duplication if it were so arranged that the immortal daughter cell always receives the DNA molecules which have the older of the two parental strands (that is, the rightmost molecules in Fig. 2*a*) and the mortal daughter always collects the molecules with the younger parental strand (that is, always collects the mistakes made in the previous generation). This rule would therefore maintain an "immortal strand" through successive generations.

To operate such a rule, the immortal strands must all be marked in some way so that sister chromatids can be distinguished at the centromere, and all centromeres must behave in a coordinated fashion. Also, if the rule is to apply to all parts of the genome and not merely to the centromeric regions, the cell must ensure that sister chromatid exchanges are rare.

In addition, if the fate of the two daughters of any immortal stem cell is determined by their position in space, the rule requires that each stem cell must enter mitosis with its duplicated chromosomes correctly aligned with respect to its surroundings. Up to a point this is certainly true. For example, in skin the plane of division of the basal cells is so arranged that both daughter cells initially lie side-by-side in the basal layer; further, there is some evidence that the successive mortal daughters of each stem cell lie alternately on one side and then on the other side of an unchanging plane of division[8]. In fact, such a pattern would fit nicely with the observation that when a given chromosome is followed through repeated rounds of duplication, in a cell forced to undergo endoreduplication by being grown in colchicine, each parental strand is found to have segregated first to one side of the plane of division and then to the other (Fig. 2*b*)[11].

Table 1 Cancer incidence in Denmark, 1943–67

Type	Commonest sites	Total cases	%
Carcinomas			
External epithelia	Skin, large intestine, lung, stomach, cervix	168,591	56
Internal epithelia	Breast, prostate, ovary, bladder, pancreas	110,182	36
Sarcomas and leukaemias		23,801	8
	TOTAL*	302,574	100

These figures are drawn from three reports that together summarise the experience of the 4.5 million inhabitants of Denmark over a period of 25 years (ref. 5). They were chosen here because, unlike most national registries, the Danish Cancer Registry reports separately for each site the number of carcinomas arising in the epithelial (parenchymatous) cells and the number of sarcomas arising in the supporting cells.

* This total excludes about 10,000 cases in which the exact site of the primary cancer was not known.

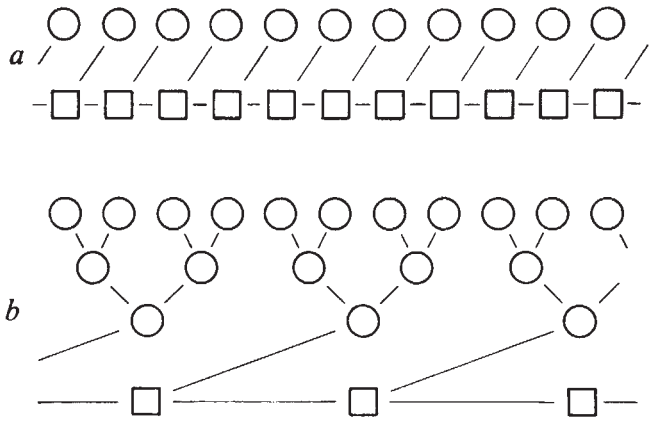

Fig. 1 Two possible programmes for the renewal of an epithelium by multiplication of the cells in the deepest (basal) layer, *a*, Renewal simply by regular, asymmetric division of stem cells; *b*, renewal by asymmetric division of the stem cells, plus some further division of the cells destined to be exported to the surface. The immortal stem cells are shown as squares and the mortal, differentiating cells as circles.

Unlike the matter of stem cell lineages which is reasonably well understood, this speculation about the relationship between stem cell lineage and DNA lineage is completely unsubstantiated. In many instances, cells labelled in their DNA during one cycle have been shown to divide this label unequally among their grand-daughters[12], and there is one example of a cell with several chromosomes (the germinating spore of *Aspergillus*) which regularly segregates the DNA strands in these chromosomes as if they are parts of a single DNA duplex[13]. It remains to be shown, however, that epithelial stem cells behave in this way. The test, of course, is to see whether stem cells can be permanently labelled in their DNA at the time they are first formed (that is, can be shown to have a DNA strand that is immortal) or alternatively to see whether they retain the label only for one generation when labelled in an adult animal (that is, normally discard the newer of their

parental DNA strands). Experiments of this kind are not yet completed.

Compartmentalisation and the natural selection of fitter variants

Having discussed two arrangements that would reduce the effective mutation rate in multicellular animals, we can now turn to a factor influencing the selective forces operating on mutant cells.

Most experimental cancers are formed only after repeated or prolonged exposure to a carcinogen. Similarly, most human cancers appear in old age, as if they usually arise as the end result of a lifetime of carcinogenesis. For this reason it is generally believed that several steps are required to convert a normal cell into an uncontrolled, invasive variant. The exact number of steps could be determined from the relationship between age and incidence provided that all steps occur at the same, unchanging rate[14,15]. If, however, the first step increases the survival of the altered cell so that this cell type steadily increases its number at the expense of normal cells, there will be a corresponding steady increase in the chance that somewhere there is a cell undergoing the next step, simply because with time there will be more and more first-step cells at risk[16,17]. So, whatever the number of restraints that must be overcome to convert a normal cell into a cancer cell, the risk that this occurs will be much increased if there are intermediate states with high survival advantage and if these variants are in fact able to displace their neighbours. We may therefore expect to find fast-multiplying tissues arranged in such a way that neighbouring stem cells (or sets of stem cells) are restricted to limited territories so that they cannot easily compete with each other.

The epithelium of the small intestine, which is the site of the most rapid cell turnover in the body, is an example of just such an arrangement. Cell multiplication is confined to indented regions of epithelium (the crypts) that project deep into the underlying mesodermal tissues. Between each neighbouring pair of crypts the epithelium forms a fold (the villus) that projects into the lumen of the gut. As a result of the continuous cell division in the crypts there is a continuous flow of cells from each crypt to the tips of the neighbouring villi, and from there the cells are shed into the gut[18]. Apparently each cohort of cells moves *en bloc* up the villus, propelled by the pressure of new cells being formed in the crypt. No matter how many immortal stem cells are normally present in each crypt (and the number is not known), any variant stem cell with high survival advantage should quickly exclude normal cells from its crypt. Unless this variant is, however, able to alter the whole structure of the epithelium (for example, by invading the surrounding mesoderm) the only way it can reach neighbouring crypts is by moving down the other sides of the villi, against the continual flow of normal cells arising in these crypts.

We see therefore that the arrangement of the intestinal epithelium into crypts and villi will tend to limit the expansion of any population of fitter variants that might otherwise form an ever-increasing target for further mutational steps. Other epithelia are no doubt partitioned in other ways. In each case the rules governing the territoriality of the proliferating cells will determine which kind of variants are the most successful.

Mutation selection and carcinogenesis

Restricting the number of stem cells, imposing a special pattern of segregation of DNA strands, and restraining the opportunities for competition between cells, are just three of the possible ways that may have been evolved in long-lived multicellular animals for diminishing the rate at which mutant cells accumulate. Of course, the rate would be further decreased if stem cells have unusually error-free forms of DNA synthesis and repair, perhaps in conjunction with a prolonged interval between successive divisions to allow ample time for any necessary repairs. The object of this article, however, is not to produce a list of all the factors that might affect mutation rate, but to show how the organisation of the self-renewing tissues of multicellular animals may often determine the sequence of events during carcinogenesis.

We have seen that the lineage of epithelial cells is so arranged that rapid renewal is normally achieved by rather few divisions of a special minority population, the immortal stem cells, which have a somewhat protected position deep in the epithelium and may, in addition, retain specially preserved DNA strands. The rate of accumulation of mutations in such a system will be proportional to the rate of division of the stem cells or perhaps, more specifically, to the rate at which they have to multiply to replace lost stem cells (that is, the rate at which new immortal strands have to be created). Anything that accelerates either of these processes could be carcinogenic. For example, cigarette smoking causes a thickening of the bronchial epithelium and obviously disrupts the ordered programme of cell renewal[19,20]. Judging from the relationship between incidence and age and duration of smoking[21], it seems that the smoker accumulates in each year about the same risk that 50 non-smokers accumulate collectively; alternatively, since incidence increases as the fourth power of time, it could be said that the smoker's bronchial epithelium goes through time at about 2.7 times the normal rate. It is tempting therefore to ascribe the increased risk of lung cancer simply to a 2.7-fold increase either in the rate of division of stem cells in the bronchial epithelium or in the rate at which

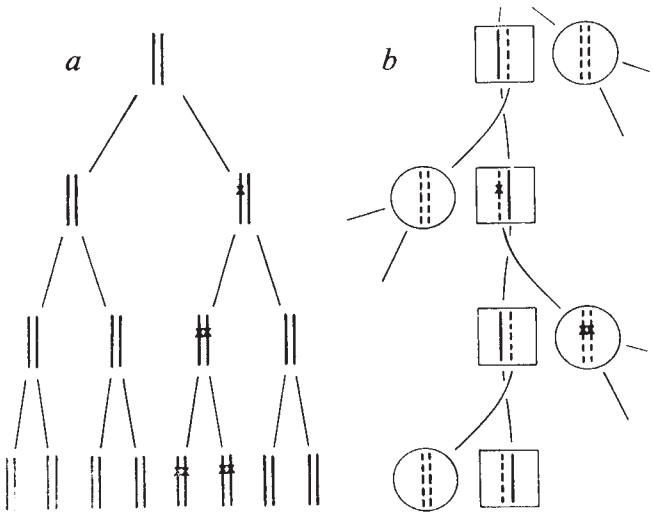

Fig. 2 The lineage of DNA undergoing semi-conservative replication. *a*, The segregation of a mutation, X, that arose as a copying error during duplication. *b*, Rearrangement of this lineage to fit the observation that parental strands apparently tend to segregate alternately to the right and left of the plan of division[11] and that, in one instance at least, the stem cells also move at division alternately to one side and then to the other[8]. The hypothesis therefore is that stem cells (□) keep the same parental DNA strands through successive cycles.

these stem cells have to be replaced. This example shows that although it is customary to think of carcinogens as necessarily acting directly as mutagens, some could act indirectly simply by altering the programme of division of immortal stem cells.

Nevertheless there is no escaping the fact that the most powerful experimental carcinogens are intense mutagens. So we should now consider what general classes of mutation are likely to lead to cancer. In particular, because most forms of human cancer seem from their age-incidence to be the end-result of several consecutive, independent steps (mutations) we should be especially interested in those mutations that are likely to affect the subsequent rate of formation and selection of additional mutations. The previous example showed how a relatively slight change in the rate of stem cell division may have a great effect on cancer incidence. Obviously the same effect could be produced by an increase in the mutation rate per cells division, resulting from change in a gene with a product concerned with the duplication or repair of DNA. Unless such mutagenic mutations confer some survival advantage, however, they will remain confined to the stem cells in which they arise, and so only if they produce enormously increased mutation rate will they raise significantly the overall mutation rate; for a single cell to double the probability that an organ with 10^8 stem cells has experienced an event which increases as the fourth power of time, that cell would have to move through time (that is, collect mutations) at 100 times the normal rate. Probably more important, therefore, are mutations that affect the interactions of a cell with its neighbours. Any mutation that gives a stem cell the ability to move out of its compartment in an epithelium may cause it to form an expanding clone of stem cells, and because such a clone would be the site of an unusually rapid rate of formation of immortal strands it should show a much higher mutation rate than the rest of the epithelium; the first mutation would therefore be acting as a 'primer' for whatever succession of further alterations are needed to make a fully invasive cancer. The most carefully studied precursor states for any human cancer are the various precancerous abnormalities of the cervix, and they show exactly the properties we would expect of primer mutations. They are characterised by the appearance of extensive clones of cells[22,23] with deranged programmes of cell renewal[24,25]; further, although for obvious reasons the undisturbed natural history of these states is not well quantitated, it does seem that the successive levels of abnormality succeed one another with a rather high probability[26,27], as if the first level of abnormality has indeed raised the rate at which mutant cells accumulate.

If stem cells do preserve an immortal DNA strand through successive divisions, the only time when it should be easy to create a primer mutation (that is, immortalise an error) may be when these immortal strands are being synthesised—that is, when the epithelium is being created or is having to regenerate. It is therefore significant that carcinoma of the cervix usually arises immediately next to the boundary with the cervical canal, where the stratified epithelium of the cervix changes to the columnar epithelium of the canal[28]. The exact position of this boundary is known to fluctuate, and it is easy to imagine that the epithelium here is especially at risk because the local population of each kind of stem cell (stratified and columnar) has to rise and fall with each fluctuation.

Most epithelia probably establish their stem cells *in utero*[29], and so, if undisturbed, are unlikely to form primer mutations. One obvious exception, however, is mammary epithelium which proliferates after puberty with each ovarian cycle[30,31] and further proliferates during each successive pregnancy. In this special case a most interesting relationship is found between the extent of the proliferation that occurs during sexual maturity and the probability that cancer develops in old age[32]. The overall (lifetime) risk of cancer developing in childbearing women seems to be linearly related to the length of the interval that elapsed between puberty and the first full-term pregnancy, rising in value from 30% to about 130% of the value for women who never have children. So there are two unusual features to be accounted for: although the mammary epithelium proliferates at each successive pregnancy, it is only the first pregnancy that affects the risk of breast cancer; second, although the incidence of most cancers (this one included) rises as about the fourth or fifth power of age, the total lifetime risk of getting this cancer proves to be directly proportional to the length of one particular time interval. The explanation could be as follows. First, we suppose that the stem cells of breast epithelium increase in number at the time of puberty and then undergo a certain fluctuation in number with each ovarian cycle until the first pregnancy, whereupon the population builds up to a final level that is no longer influenced by the ovarian cycle or by additional pregnancies. Second, we suppose that the chance of a primer mutation occurring is simply proportional to the total number of times that immortal strands have had to be made in this epithelium (that is, the number of stem cell generations that have occurred)—much in the way that the frequency of mutants in a bacterial chemostat increases linearly with time[33]. Once pregnancy establishes the final unchanging population of stem cells, the epithelium becomes as protected against mutation as any other proliferating epithelium.

For obvious reasons, experimental carcinogenesis is usually studied in systems where brief exposure to a carcinogen produces a high incidence of cancer within a few weeks or months. It could be argued that such systems, using very strong carcinogens, are not suitable models for human cancer which typically arises at a slow rate after an incubation period of many years. Therefore this article, being on the natural history of cancer, has used examples of the common human cancers. The idea that special primer mutations are the first step in the formation of most cancers comes in large part, however, from certain reports of experimental carcinogenesis where the process could be divided into the separate stages of 'initiation' and 'promotion'[34,35].

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