

In considering tumorigenesis, much attention is paid to genome instability and mutation rates. While reflecting on the circumstances that have led to this emphasis on mutation rates, Ian Tomlinson and Walter Bodmer point out that an increased mutation rate does not necessarily cause a tumor to grow and that selection is in fact the mechanism that drives the cellular, somatic evolution that leads to cancer.

Selection, the mutation rate and cancer: Ensuring that the tail does not wag the dog

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Recently, studies of somatic cancer genetics have tended to focus on the conditions that may be favorable to tumor growth, rather than on the forces and processes that directly promote tumorigenesis. As a result, genomic instability—in other words, a raised intrinsic mutation rate—has come to be regarded as the most important factor in tumorigenesis. Thus, for example, BRCA1 and BRCA2 mutations have been interpreted in terms of the association of the respective proteins with DNA repair pathways, rather than in terms of any effect on cell proliferation or death¹. There are several reasons for this emphasis. One is a deficiency of ‘classical’ or population geneticists studying somatic cancer genetics². Another is the existence of rare syndromes, such as hereditary non-polyposis colon cancer³ (HNPCC) and ataxia telangiectasia⁴, that are associated with raised mutation rates and cancer predisposition and have recently been the subjects of major advances in cancer genetics. Many researchers have therefore turned to theoretical discussions of workers such as Loeb, who has long promoted the central role of the mutation rate in tumorigenesis^{5,6}.

There are, however, overriding reasons to believe that the current focus on genomic instability in cancer is misplaced, simply because a raised mutation rate does not itself cause a tumor to grow. The fundamental force for change has been neglected. Tumorigenesis has long been realized to be an evolutionary process, starting with the writings of Boveri, James Murray, Little and Tyzzer early this century and continuing with workers such as Cairns⁷. The most powerful evolutionary force is natural selection, which acts directly to increase the frequency of advantageous alleles in the population. Selection is also the driving force behind tumor growth. A raised mutation rate may make evolution (or tumorigenesis) faster, but is not necessary for evolution (or tumorigenesis) to occur.

Colorectal cancer is probably the best model system for analyzing the roles of selection and genomic instability in tumors, because the accessibility of tumors to resection within the bowel lumen has allowed the stepwise nature of their progression to be relatively well defined in both histological and genetic terms. About 15% of sporadic colorectal cancers show microsatellite instability, which may reflect an increased intrinsic mutation rate, owing to defective mismatch repair (MMR). The growth of most colorectal cancers starts with a dysfunctional mutation at the adenomatous polyposis coli (APC) locus, soon followed by a second change eliminating completely normal APC functions. This results in an adenoma, which may then progress to carcinoma. Mathematical models show the overwhelming importance of selection in the growth of colorectal tumors: Tumorigenesis almost always starts with a normal mutation rate⁸, because mutations at APC have a much greater chance of occurring before two MMR mutations

have occurred. Molecular analysis of the spectrum of APC mutations in colorectal cancers and publicly available cell lines bears out the conclusions of the models.

The spectrum of APC mutations in tumors with microsatellite instability does not reflect an underlying defect in MMR (for example, frameshift mutations or changes at short repeats) (ref. 9), showing that the APC mutations occurred while MMR was normal. In HNPCC, in contrast, one MMR mutation is inherited, and tumorigenesis may start with a raised mutation rate^{9,10}, because a ‘second hit’ at the MMR locus can occur before even the ‘first hit’ at APC. There is no evidence that APC mutations act by any mechanism other than selection for some sort of growth advantage. The relative powers of a selective advantage and an increased mutation rate are illustrated by the fact that HNPCC patients develop about the same number of colorectal tumors as the general population (although they develop more cancers; see below), whereas FAP patients (with germline APC mutations) usually develop thousands of colorectal tumors³.

After two mutations at APC, expansion of the colorectal tumor clone occurs¹¹. Usually, this will occur fast enough to prevent any requirement for the tumor to have a raised mutation rate for the next selected mutation—probably *K-ras*—to occur⁸. Subsequent rounds of clonal expansion, mutation and selection repeat this process, leading to the stepwise progression of colorectal tumors. It has been argued¹² that mathematical models⁵ have shown that the number of mutations found in typical cancers is too great to be explained without increased mutation rates, but this interpretation is erroneous. The calculations on which the argument is based do not constitute a formal mathematical analysis, and the model does not take into account clonal expansion¹³. Given not only the existence of clonal expansion, but also the fact that the rate of cell turnover probably far exceeds the net rate of tumor growth (thus increasing the number of cell divisions per unit time), we do not believe that any assumption of a raised mutation rate is necessary to explain carcinogenesis in general⁸ or its specific features, such as the number of mutations observed in cancers⁶. The whole intestinal epithelium replaces itself about every 2 to 3 days, representing more than 100 cell generations per year, or at least 2,000 during the usual 20-year progression of a colorectal carcinoma. One of our models, furthermore, predicts the existence of several finite increases in cell number before exponential growth is achieved¹⁴. Each new equilibrium may persist for many cell generations before the next mutation giving a further advantage arises and is successful. This situation allows plenty of scope for mutation at normal rates, followed by selection, not only to explain carcinogenic progression as an evolutionary process driven by selection, not mutation, but also to account for long lag periods without

having to assume very slow exponential growth rates or improbable conditions of dormancy.

An arguably neglected factor is the disadvantage to a cell of a raised mutation rate, particularly in early tumors. Indeed, much cancer therapy is based on the principle that cells with a high mutational load tend to undergo apoptosis, and there is supporting evidence for this from colon cancer cell lines, according to our interpretation of these data¹⁵. More subtle disadvantageous effects of a raised mutation rate may also occur. Tumorigenesis in HNPCC, for example, may initially progress relatively rapidly owing to defective MMR: the small-scale mutations that occur can be tolerated by the growing tumor, although they may lead to an accentuated immune response as evidenced by the common loss of HLA Class I expression, mostly due to $\beta 2$ microglobulin mutations¹⁶. HNPCC cancers seem then to become trapped in an 'evolutionary cul-de-sac'. For reasons unclear at present, HNPCC tumors do not acquire the gross aneuploidy characteristic of most late-stage colon cancers, and thus even late-stage colorectal cancers in HNPCC progress slowly and have a good prognosis.

Some colorectal cancers undoubtedly do end up with a raised mutation rate, whether in the form of defective MMR, a tendency to aneuploidy or some other type of genomic instability. No colon cancer syndrome is directly comparable with the recessive syndromes, such as xeroderma pigmentosum, in which there is a pre-existing raised mutation rate and tumors of specific sites grow more frequently. The tumors of HNPCC patients almost always acquire a raised mutation rate, because one defective MMR allele is inherited, and somatic mutation, allele loss or methylation of the other allele is acquired relatively easily. In some sporadic colorectal cancers, genomic instability may simply result from chance: the selected clone may acquire mutations at a DNA repair locus, for example; these mutations may initially 'hitch-hike' with mutations selected for a replicative advantage, but the DNA repair mutations may later provide faster tumor evolution through genomic instability. We predict that in sporadic colorectal tumors, genomic instability, when it arises, is more likely to be a feature of later-stage tumors at a time when the mutation rate is in any case less limiting because of increased population size, although there may be other constraints on tumor growth. There is experimental evidence to support this prediction¹⁷.

Maybe, however, these are not mutually exclusive in colorectal cancer. There is conclusive evidence for an intimate association between the cell cycle and DNA repair. Mutations of a single gene can therefore both prevent cell-cycle arrest—that is, they are selected for an effect on cell proliferation or decreased cell death—and cause an increased mutation rate. Indeed, one might anticipate a 'runaway' process whereby an initial defect in the response to DNA damage means that a raised mutation rate is no longer disadvantageous, leading to the tolerance of more mutations, more defective DNA damage response and so on. If the principle is accepted that mutations of one gene may primarily cause a selective advantage and secondarily lead to an increased mutation rate, there are many examples of candidates: p53, ATM, BUB-1, BRCA1, BRCA2 and even the MMR genes¹⁸ are all likely to have such dual roles. The proposed gatekeeper–caretaker distinction between different types of cancer genes¹⁹ may need adjustment to include

'gatetakers' or 'carekeepers'.

It is striking that excision repair mutations rarely occur in sporadic cancers, despite leading to an increased cancer risk through genomic instability when two mutant alleles are present in the germline. This fact alone means that there is no simple association between increased mutation rates (or genomic instability) and tumorigenesis⁸. Mutations that provide a selective advantage are essential for tumorigenesis; genomic instability is likely to be important in some situations and in some tumors, but it is neither a general driving force behind tumorigenesis nor essential for tumor growth. Cairns² has written that "unlike classical geneticists, who are more interested in genetic mechanisms and natural selection than in mutagenesis, somatic geneticists tend to be concerned with mutagenesis rather than the selective processes that may operate in somatic tissues." We would wish to persuade "somatic geneticists" to consider selection before turning their attention to mutation. Selection is surely the overriding mechanism of cellular, somatic evolution leading to cancer, just as it is for evolution, 'à la Darwin', at the organismal level. This view of carcinogenesis can, we believe, lead to considerable new insights into the carcinogenic process.

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