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Endogenous mutagenesis and cancer

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Abstract

Mutations in DNA accrue relentlessly, largely via stochastic processes. Random changes accumulate, eventually disabling genetic components which result in the formation of the cancer phenotype. Given the infrequency of measured nucleotide changes and the requirement for several mutations to occur in the same cell, it has been postulated that the rate of mutation must become elevated early in the course of evolution of the cancer. Recently, large scale sequencing of tumor DNA has sought to directly measure random mutations. We discuss the implications of these findings and the factors that must be considered in order for fruitful determination of whether a mutator phenotype is a necessary precursor for cancer.

Keywords: Mutagenesis; Mutator; Stem cell; Cancer

1. Introduction

The concept that cancers exhibit a mutator phenotype early during tumor progression emanates from studies on infidelity of DNA synthesis by purified DNA polymerases and on the induction of transient hypermutable states in bacteria as a result of DNA damage. With the discovery of the structure of DNA and the specificity of base-pairing it was assumed that the nucleotide sequence in DNA was inviolate and would be passed intact from one cell generation to the next without error. Bryn A. Bridges is one of a handful of scientists who realized that the nucleotide sequence of DNA was governed by homeostatic mechanisms that balanced DNA damage with DNA repair. Among his contributions are studies on the mechanisms for the fidelity of DNA synthesis and investigations on error-prone DNA synthesis that in bacteria constituted the SOS response. Bridges et al. [1] and Witkin [2] provided evidence that the gaps in DNA caused by UV-damage could be resynthesized by an error-prone repair process. He continually emphasized that the hypermutation in bacteria under stress should guide us in understanding transient states of hypermutagenesis that might occur in human pathologies and cancer in particular. At Gordon Conferences his hiking ability and surfing were legendary and his presentations were delivered with precision in Shakespearean eloquence. He frequently considered different models to explain the molecular basis of mutagenesis and the accumulation of mutations in bacteria during stationary growth. In choosing a topic for this commemorative issue it seemed appropriate to consider the concept of a mutator phenotype in cancer and moreover to emphasize arguments against this concept. Bryn A. Bridges enjoys controversies in science.

2. Mutations in cancer

With few exceptions, all tumors show genomic instability. This instability can manifest itself as changes

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in the nucleotides sequence of DNA, such as base substitutions, insertions, deletions, as well as expansions or contractions of microsatellite repeats, or in larger genetic alterations, such as aneuploidy, chromosomal translocations and gene amplifications [3]. As a result, we have hypothesized that human tumors contain thousands of somatic mutations. The presence of large numbers of random mutations within a tumor has important consequences with respect to therapy and prevention [4]. What is not known is whether genomic instability reflects a mutator phenotype that is a necessary prerequisite for tumorigenesis. Clearly a mutator phenotype is a sufficient prerequisite, as exemplified by a variety of cancer-prone syndromes resulting from genetic defects in DNA repair pathways. Examples include defects in mismatch repair resulting in a familial colorectal cancer syndrome (human non-polyposis colorectal carcinoma) [5], nucleotide excision repair resulting in skin cancer (xeroderma pigmentosum) [6] as well as homologous recombination and double strand break repair, associated with breast carcinoma and lymphomas (BRCA1/BRCA2, ataxia telangiectasia) (reviewed in [7]). Mouse models have provided further evidence that mutations that obliterate DNA repair [8] and mutations that reduce the fidelity of DNA polymerases [9] result in cancers in multiple tissues. If a mutator phenotype is required before a cell can progress to cancer, then this commonality might be exploited to detect many human cancers at a point very early in tumor development.

3. The mutator phenotype hypothesis

Given the infrequency of mutations in normal cells, it has been suggested that normal mutation rates are insufficient to explain the multiple mutations found in tumors including those in tumor suppressor genes and oncogenes [3]. Others claim that normal mutation rates are sufficient when a growth advantage is conferred over time to the growing tumor clone [10]. The debate as to whether a mutator phenotype is necessary for tumorigenesis continues, mainly because of the technical difficulties associated with detection of mutation rates in human tissue. Central to the issue is the theoretical estimate for the number of generations the progenitor cancer cell undergoes prior to the clinical detection of a tumor. In principle, the more somatic cell divisions that a potential cancer cell undergoes, the lower the mutation rate needed to account for the multiple mutations observed in a tumor. The actual number of divisions any particular stem cell goes through in the lifetime of an individual is not accurately known and is likely to vary amongst different tissues. Moreover, the number of generations that occur during tumor progression is not established. The estimates vary, along with the necessity to invoke a mutator phenotype.

4. In defense of the mutator phenotype

Recently, Wang et al. [11] have attempted to determine the mutation frequency of nucleotide changes in colorectal tumors using a brute force sequencing approach. Analysis of approximately 3.2 Mb of coding DNA from over 470 genes revealed 3 somatic mutations, corresponding to a mutation frequency of 1 alteration per Mb of tumor DNA. The authors conclude that this frequency is consistent with what is expected given current estimates for normal nucleotide mutation rates, and estimates for colorectal stem cell turnover. They conclude that most sporadic colorectal cancers do not display a mutator phenotype at the nucleotide level. Let us consider the arguments that may limit this conclusion. Firstly, a mutation frequency of one mutation per 1 Mb must be considered with respect to the frequency of mutations in DNA obtained from normal tissue. Since no normal DNA was sequenced, except to verify the presence or absence of mutations, the control frequency remains unknown, and the authors rely on theoretical estimates of mutation rates in normal cells. Secondly, the tumor cells themselves were cultured in nude mice and in some cases cell culture, which may have subjected the tumor cells to some form of selection pressure or altered mutagenic environment. Thirdly, only non-synonymous DNA changes were assessed and only in coding regions from genes that remain after LOH of the other allele. Given that only single copies of these genes remain, there is the likelihood that mutations resulting in loss of function would have been selected against during evolution of the tumor and thus would have not been detected. Most importantly, the DNA used for sequencing was obtained after amplification of 5 ng or approximately 1500 genome equivalents of tumor DNA by PCR.

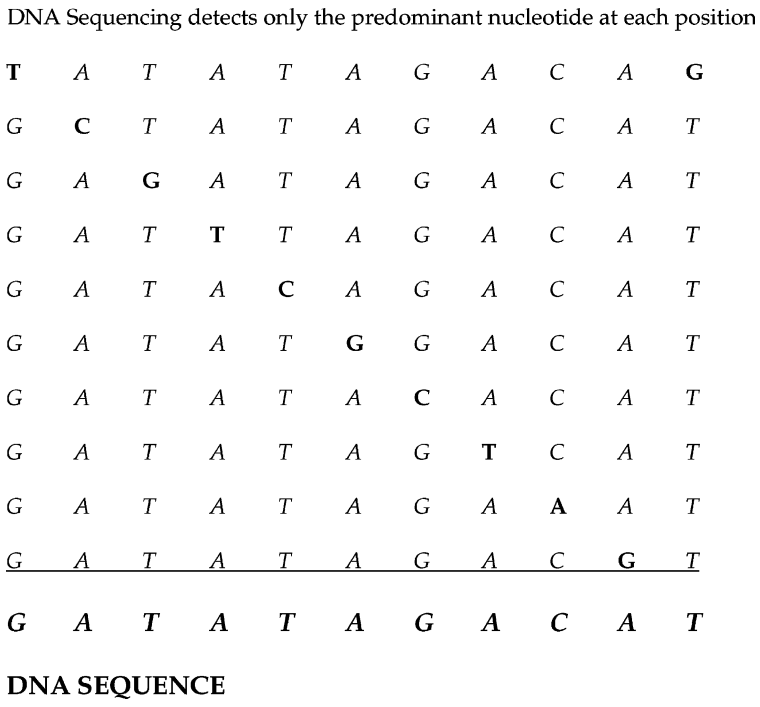

Fig. 1. The sequence obtained from these 10 different clones is not actually present in any of them. DNA sequencing will reveal the most prevalent species. Random substitutions that are present in less than 10% of the nucleotides at each position would not be detected.

DNA sequencing starting with a heterogenous population only detects the most frequent substitutions at each position. As a result, random substitutions that are present in less than 10% of the nucleotides at each position would not be detected. Fig. 1 illustrates this point. Here the sequencing readout, GATATAGACAT results, even though this sequence is not actually present. In effect, the sequencing data obtained from PCR of multiple genome copies only shows the most abundant mutations present in the tumor. Thus, the only substitutions that would be detected are those that are present in the majority of clones; substitutions that did not result in clonal proliferation would only be detected if they occurred during the first few tumor cell divisions. If the predestined cancer cell arose from a field of normal cells experiencing elevated mutation rates, then the tumors that are derived from these stem cells should possess an elevated mutation frequency, even with the PCR methodology employed by Wang et al. The question then becomes, does the measured frequency of one mutation per Mb of DNA indicate an *early* mutator phenotype or not? The estimated rate of stem cell divisions thus becomes the most important factor.

Recently, Tomlinson et al. [12] have put forth the view that after 50 years of adult life, every normal colonic stem cell will have accumulated more than 125,000 mutations, or roughly 21 mutations per 1 Mb. This surprising estimate is based on an endogenous mutation rate of 5×10^{-9} mutations per nucleotide per cell division and 5000 stem cell divisions after 50 years. Both Wang et al. and Tomlinson et al.'s theoretical calculations for the endogenous mutational frequency in a human colon cell depend upon the assumed rate of 100 divisions per year or 5000 divisions over 50 years of the ultimate stem cells whose progeny repopulate the entire colonic epithelium. However, the majority of these estimates were extrapolated from repair kinetic studies of the mouse small intestinal crypt following high doses of ionizing radiation [13–15], and may be different from the human colon in the kinetics of epithelial replacement. Much

lower estimates for stem cell division have been postulated. A hierarchical model in which a colonic stem cell is no more than 55 generations from the zygote has been advanced by Morris [16]. In addition, the recent discovery of the plasticity of circulating stem cells, raises the possibility that stem cells from a variety of tissues may be replaced from a circulating pool of pluripotent cells [17,18]. On top of these factors, each of which would significantly lower the estimated number of divisions that a stem cell may have experienced, is the tantalizing possibility that stem cells themselves might have much lower mutation rates per se, than other somatic cells, by a process in which older template strands are segregated into the originating stem cell. This notion that stem cells may preferentially segregate their parental DNA following DNA replication was first proposed by Cairns and coworkers [19] and has been further elaborated by Potten et al. [20]. If it is correct that colon stem cells can indeed accumulate so many mutations, one then wonders about other stem cells that do not undergo so many divisions, yet can give rise to malignancies.

A definitive understanding of the human stem cell generations in the colon is lacking, and estimate range from tens to thousands of cycles of regeneration over the lifetime of an individual. In short, the question of the necessity for a mutator phenotype remains open until mutation frequencies are determined for both normal and tumor DNA from a variety of cancers by methods that can detect random substitutions.

5. The quest continues

Most adult cancers are epithelial, originating in tissues with a high proliferative index in contrast to tissues with low mitotic indices such as cardiac myocytes and CNS neurons. Conditions that lead to constant cell renewal, such as tissue damage from chronic inflammation including inflammatory bowel disease and Barrett's esophagus, for example, also predispose cells to cancer. Although the effects of an increase in cell division cannot be dissociated from the genotoxic effects of the inflammatory environment in these cases, it is possible that the degree of elevation of mutation rate required early during tumor progression differs depending on the capacity of a tissue for self-renewal. Those tissues with the capacity for high levels of self-renewal may require only a modest increase in mutation rate compared to tissues that have lower mitotic rates.

Eventually, mutation accumulation may be detrimental and the cancer may no longer express an elevation in mutation rate. However, the footprint of its evolutionary history, the increased mutation frequency, is not erasable. Large scale DNA sequencing of random mutations from DNA of isolated tumor clones, will eventually provide the answer to the debate concerning whether most tumors have an increased frequency of random mutations compared to normal tissue. But as we have seen, careful attention must be paid to how the DNA is procured for sequencing, what tumors are selected and what regions of DNA, coding or non-coding, are chosen to ensure that indeed the mutations measured are random and therefore indicative of a mutator phenotype.

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