A Mutator Phenotype in Cancer

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Abstract

We have proposed that an early step in tumor progression is the expression of a mutator phenotype resulting from mutations in genes that normally function in the maintenance of genetic stability. There is new and strong experimental evidence that supports the concept of a mutator phenotype in cancer. As technologies for chromosomal visualization and DNA advance, there are increasing data that human cancer cells contain large numbers of mutations. First, I will review the concept of a mutator phenotype. Second, I will present the recent evidence that individual cancer cells contain thousands of mutations. Third, I will explore potential target genes that are required for maintenance of genetic stability in normal cells and ask if they are mutated in cancer cells. Fourth, I will address the timing of a mutator phenotype; is it an early event during tumor progression? Do tumors already contain cells that harbor mutations rendering them resistant to most chemotherapeutic agents? Lastly, I will speculate on the theoretical and practical implication of a mutator phenotype in cancer and consider the possibility of cancer prevention by delay, i.e., a reduction in mutation rates early during carcinogenesis might slow the progression of tumors.

Introduction

The maintenance of a species, and perhaps of an individual, requires that DNA replication be exceptionally accurate. Information contained in DNA must be accurately copied and transmitted to each daughter cell. The fact that the DNA in cancer cells is different from the DNA in normal cells indicates that carcinogenesis involves substantial errors in DNA replication, deficits in DNA repair, and alterations in chromosomal segregation.

In normal cells, DNA replication and the partitioning of chromosomes are exceptionally accurate processes. During every division cycle, each daughter cell receives a full and exact complement of genetic information. Each parental cell duplicates its genome containing approximately $6 \times 10^9$ nucleotides and then partitions the DNA equally between the two newly generated daughter cells. The fidelity of this transfer is precise in both germ-line and normal somatic cells. Errors in these processes result in mutations. In germ-line cells, a few mutations are necessary to permit a species to adapt to environmental changes. In principle, somatic cells might be able to tolerate the production of large numbers of mutations, because these mutations are not passed on to subsequent generations and thus would not accumulate within the gene pool of a species. However, when these somatic mutations are expressed, they cause diseases that curtail the life of individuals. In humans, cancer is the most prominent of these diseases. The concept of a mutator phenotype in cancer was initially formulated (1) to account for the disparity between the rarity of mutations in normal cells and the large numbers of mutations present in a variety of human malignancies. This hypothesis has evolved in accord with our increasing ability to dissect carcinogenic processes at the molecular level (1–4). It seems pertinent now to summarize recent data and to consider the theoretical and practical implications of a molecular phenotype in cancer.

There are multiple DNA synthetic transactions that could be altered in normal cells and generate a mutator phenotype; these include both errors in DNA synthesis and inadequate repair of DNA damage. Here we define a mutation as any change in the nucleotide sequence of cellular DNA. Errors in DNA synthesis are generated by misincorporation of nucleotides by DNA polymerases. Mutations are produced when the number of these misincorporated nucleotides exceeds the capacity of cells to excise and repair the lesions. DNA damage is produced by reactive molecules generated in cells by normal metabolic processes, as well as by exogenous environmental agents. Cells have evolved multiple mechanisms for the excision and repair of DNA damage. Nevertheless, the amount of DNA damage can exceed the cell’s capacity for DNA repair, and the residual nonrepaired lesions can be a dominant source of mutations during DNA replication.

Somatic mutations can also result from errors in the partitioning of chromosomes during cell division. Inequalities in chromosomal partitioning result in aneuploidy, a change in chromosome number, which is one of the most common hallmarks in cancer cells. Unfortunately, the genes responsible for chromosomal segregation are only now beginning to be identified; as a result, partitioning errors will be considered only briefly in this prospectus.

A Mutator Phenotype in Cancer

Considering the rarity of mutations in normal cells and the large numbers of mutations observed in human cancers, it has been proposed that the spontaneous mutation rate in normal cells is not sufficient to account for the number of mutations found in human cancers (1). Simply stated, cancer cells exhibit a mutator phenotype. This phenotype is the result of mutations in genes that function in the maintenance of genomic stability. It is manifested by increases in mutation rates and in genetic evolution of cancer cells that drives tumor progression.

Historical Perspective

The multiplicity of chromosomal aberrations found in many human tumors was noted by pathologists more than 100 years ago and has served to identify malignant cells and to stratify the aggressiveness of certain cancers (5). Chromosomal aberrations include translocations, gene amplifications, deletions, and insertions; these can be classified as mutations that involve the repositioning, addition, or omission of millions of nucleotides. The large numbers of these alterations in certain cancers, coupled with the morphological and physiological heterogeneity of cancer cells within individual tumors, were important observations that underpin the concept of a mutator phenotype in cancer. We initially proposed that the multiple mutations found in tumor cells would result from mutations in genes that guarantee the fidelity of DNA synthesis or the adequacy of DNA repair (1). Mutations resulting in errors in nucleotide incorporation or the capacity of the cell to repair DNA lesions will promote the appearance of chromosomal aberrations.
tions in bacterial DNA polymerases were known to produce mutations throughout the genome (6). Individuals with rare inherited diseases such as xeroderma pigmentosum (7) or ataxia telangiectasia (8) exhibit a high incidence of certain cancers, and cells from these individuals exhibit decreased survival upon exposure to the same agents that cause cancers in these individuals. Despite the presence of multiple “hot spots,” DNA damage is primarily a random process; if the damage is not repaired, it could serve as a potent source of mutations. By chance these mutations might be in genes required for the maintenance of genetic stability. Mutations in genetic stability genes could produce additional mutations throughout the genome. Among these would even be mutations in other genetic stability genes, leading to a cascade in mutations as cancers evolve.

At about the same time, Nowell (9) proposed that a mutator phenotype in cancer is the result of repetitive rounds of clonal selection in which mutations in a single cell impart a selective growth advantage, allowing its progeny to proliferate and populate the tumor. Tumor progression results from sequential proliferation of more aggressive sublines. Successive rounds of clonal expansion drive tumor progression and result in multiple mutations. It seems likely that both mechanisms contribute to the multiple mutations found in cancer; but surprisingly, the two mechanisms could act in synergy. Mutation accumulation and clonal selection may be inextricably linked (see below).

 Mutations Are Infrequent in Normal Human Somatic Cells

Normal human cells accurately replicate DNA every time they divide. The overall mutation rate in somatic human cells has been estimated at $1.4 \times 10^{-10}$ nucleotides/cell/division or $2.0 \times 10^{-7}$ mutations/gene/cell division (10). Considering that each cell contains $\sim 70,000$ genes, we estimate that each cell accumulates one mutant gene during the life span of an individual (3). However, we lack information on mutation rates in somatic cells in different tissues and in different mammalian species. It is likely that somatic mutation rates in human cells are lower than in rodent cells, and this may contribute to the resistance of human cells toward transformation in vitro (11, 12). If this disparity between humans and rodents is manifested in cancer precursor cells, it suggests that carcinogen testing in rodents is not an adequate model for human susceptibility, although it is currently the best available.

A large body of evidence indicates that most tumors originate in one or a few stem cells. These cells have a remarkable plasticity, allowing them to differentiate into a variety of cell types, including those that are malignant. On the basis of the assumption that the accuracy of DNA replication in stem cells is similar to that in human somatic cells, we estimated that each stem cell amasses one to two mutant genes (assuming 100 cell divisions during a human life span; Ref. 13). However, based on a Poisson probability distribution, there would be a few stem cells that would contain as many as 12 mutations. The slope of the exponential increase of cancer as a function of age suggests that there are 6–12 cancer-causing events (14, 15), each of which is rate limiting for tumor progression. If one assumes that these rate-limiting events are mutations, then it is conceivable that normal mutation rates can account for the age increase in cancer. Each of these mutations would have to offer a selective growth advantage, even if it occurred on a single allele. However, if we restrict our analysis to genes that have been shown to be associated with human cancers (an estimated 100 known oncogenes and tumor suppressor genes; Ref. 16), then the normal mutation rates would produce only two or three mutations in cancer-associated genes. In either case, the mutation rate in normal cells cannot account for the thousands of mutations being found in human cancer cells.

Multiple Mutations Are Frequent in Human Tumors

Historically, cytological observations on variations in chromosome number and integrity in cancers have always implied that cancers contain multiple mutations. In only rare instances are specific chromosomal aberrations diagnostic of particular malignancies. More informative cytogenetic methods that are being developed may reveal additional examples of tumor-specific chromosomal abnormalities. Nevertheless, the majority of chromosomal abnormalities are not tumor specific but instead may indicate the manifestations of an underlying instability in cancer cells.

Chromosomal Instability

Chromosomal aberrations in cancer cells have been recognized as a criterion for cancer and for grading tumors to predict prognosis. With our increasing ability to characterize chromosomes at the molecular level, there has been a expanding literature on multiple chromosomal alterations in different tumors. Two techniques have been particularly revealing:

(a) Comparative genomic hybridization measures differences in hybridization between fluorescent labeled fragments of tumor and normal DNA using metaphase chromosomes as a scaffold. A large variety of tumors have been shown to exhibit changes in DNA copy number (17). Some breast cancers exhibit as many as 15 changes in DNA copy number (18), and an even greater number were exhibited in high-grade ovarian cancers (19). Even benign tumors such as uterine leiomyomas (20) exhibit changes in DNA copy number. Comparative genomic hybridization can detect deletions or amplifications only if they are 1–10 megabases (21); presumably, with the development of more sensitive techniques, a greater number of changes may be detected in cancers. These studies are carried out using bulk DNA, and thus the only changes observed are those that occur in most of the population. However, a PCR-based strategy for global amplification of DNA from a single cell has affirmed the multiplicity of changes in DNA copy number in cancers and can be used to map clonal evolution (22).

(b) Measurements of loss of heterozygosity in tumors have been analyzed using microsatellite markers that scan only a small fraction of the genome. Nevertheless, some primary breast cancers exhibited $>20$ regions with loss of heterozygosity (23). Because the probes used in this study sampled only 0.01% of the genome, we calculate that some of these tumors might contain as many as 200,000 regions with loss of heterozygosity (24). This calculation is biased, because the number of mutations detected in small regions may not be representative of that which occurs throughout the entire genome, and also the target sequences examined were those demonstrated previously to be altered frequently in human tumors. Nevertheless, if these changes are in any way representative of other changes throughout the genome, one must conclude that in many cancers there are tens of thousands of mutations.

It has been argued that there are two types of genetic instability, microsatellite instability and chromosomal instability (25, 26). Cancers that exhibit extensive microsatellite instability are those with mutations or inactivation of mismatch repair genes; those that exhibit predominantly chromosomal instability are those with mutations that affect the partitioning of chromosomes during mitosis. However, both microsatellite instability and chromosomal instability may be manifestations of a mutator phenotype. Both of these phenomena may be easy to detect; microsatellite sequences are subjected to slippage during copying and are present in thousands of copies, and chromosomal alterations can be visualized cytologically, because they can involve millions of nucleotide units in DNA (27). In contrast, random
changes in one or a few adjacent nucleotides in DNA could occur more frequently, yet would be very difficult to detect.

**Aneuploidy**

Aneuploidy is a change in chromosomal number resulting from unequal partitioning of chromosomes during cell division. It occurs frequently in solid tumors, and the generation of aneuploidy has been proposed as the initiating event in carcinogenesis (28, 29). Aneuploidy provides a one-step process that generates thousands of genetic alterations that could destabilize the genome. However, diseases such as Down’s syndrome present a striking enigma; cells contain an extra copy of chromosome 21 with some 200 genes (30), and yet these cells differentiate to produce an individual. Although individuals with Down’s syndrome exhibit an elevated incidence of leukemia, if anything they have a decreased incidence of most solid tumors (31). Furthermore, one still has to face the proverbial dilemma of whether aneuploidy generates genomic instability, or whether genomic instability generates aneuploidy, or are both manifestations of an underlying mutator phenotype. In Barrett’s esophagus, alterations in p53, p16, and increased tetraploidy (4N) precede aneuploidy (32, 33), and genetic instability in ulcerative colitis precedes tumor detection (34).

**Microsatellite Instability**

The extensiveness of cancer-associated genetic instability was first heralded by findings of mutations in repetitive sequences. Peinado et al. (35) used random oligonucleotides as arbitrary primers in PCR reactions and observed additional products of different sizes using DNA obtained from human colon tumors compared with those obtained using DNA from adjacent normal tissues. The tumor-specific PCR products with altered lengths encompassed segments with repetitive nucleotide sequences. These investigators sampled only a small fraction of the genome, and yet within their limited vista they observed large numbers of alterations in DNA sequence. Upon extrapolating these results to the whole genome, they concluded that some tumors contained as many 100,000 mutations. The tumor cells with the greatest numbers of changes in the lengths of repetitive sequences were subsequently shown to possess mutations in mismatch repair genes (36). Altered repetitive sequences were mainly detected in segments between genes (microsatellites) and occurred predominantly in HNPCC. Mutations in repetitive sequences within exons were subsequently demonstrated (37, 38). These results were confirmed recently and extended to sporadic colon cancers using inter (simple sequence repeat)-PCR (39). These authors estimate that many sporadic colon cancers contain tens of thousands of mutations, and that many of these mutations are already present in premalignant polyps. In the case of HNPCC, instability is mediated by mutations in mismatch repair genes that function in correcting replication error. Many sporadic colon (40) and sporadic gastric (41) cancers that do not appear to contain mutations in mismatch repair genes exhibit hypermethylation of the promoter region of MLH1, thus silencing mismatch repair by an epigenetic mechanism. A large number of nonhereditary cancers have been shown to exhibit alterations in microsatellite repeats to a lesser extent but do not appear to involve mutations in mismatch repair genes. It remains to be established whether the source of this microsatellite instability in non-HNPCC tumors is the result of decreased expression of mismatch repair genes or is attributable to mutations in other genes involved in maintaining genetic stability.

The cause(s) of microsatellite instability remains to be determined. In general, mutations are generated when the rate of mutation pro-

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1 The abbreviation used is: HNPCC, hereditary nonpolyposis coli.

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length alterations in early adenomas, and additional microsatellite alterations appeared as the tumors progressed to adenocarcinomas. The differences in the spectrum of mutations in the APC gene in tumors that exhibit microsatellite instability and in tumors that do not argue strongly that mismatch repair alterations occur prior to APC mutations (48). These combined studies support the logical inference that a mutator phenotype is an early event in the generation of colon tumors in HNPCC, at least with respect to mutations in mismatch repair genes. However, Homfray et al. (50) reported no differences in the frequency or types of mutations in the APC gene between sporadic colon tumors and colon tumors that contained mutations in mismatch repair genes (HNPCC). These authors concluded the opposite, that APC mutations initiate carcinogenesis, and mutations in mismatch repair genes occur at a later stage (51). Despite the later findings, the weight of evidence would indicate that mutations in mismatch repair genes occur early during the course of colon carcinogenesis in tumors that exhibit microsatellite instability.

Origins of a Mutator Phenotype

Initial Events

The mutator phenotype hypothesis does not address the sources of mutations that initiate carcinogenesis. Epidemiological studies have identified mutagenic environmental agents that are causally associated with specific human tumors. Except for tobacco, UV irradiation, and aflatoxin B1, only a few environmental agents have been demonstrated to be causally associated with major human cancers. Most known carcinogens damage DNA, and if this damage is not repaired, it can cause mutations throughout the genome including genes that are required to maintain genetic stability. I should stress that the cause of nearly half of all human cancers has not been documented; these cancers are not geographically clustered nor associated with exposure to specific environmental agents. These cancers could arise from exposure to multiple environmental agents, each causing a small increment in mutations. Alternatively, these cancers could be “spontaneous” and emanate from DNA by endogenous reactants (57) including alkyl groups, activated lipids, metal cations, and oxygen-reactive species (58). DNA damage by endogenous reactions is similar to that caused by environmental chemicals and can also produce random mutations throughout the genome. The extent of DNA damage by normal reactive metabolites appears to be exceptionally high. It has been estimated that oxygen-reactive species generate as many as 10,000 DNA damaging events/cell/day (59). A similar number of lesions is generated by spontaneous depurination, resulting in the loss of purine bases in DNA and miscoding by the residual apurinic site (60). In total, it has been estimated that each cell in our body generates and removes hundreds of thousands of altered bases from DNA/cell/day (61). These background or “spontaneous” DNA-damaging processes might not only generate the initial lesions that confer a mutator phenotype but might also contribute to the accumulation of mutations during tumor progression, specifically in cells harboring mutations in DNA repair genes. A number of reports suggest that tumor DNA contains altered bases in DNA and that these alterations result from carcinogen exposure. The presence of chemical alterations in DNA must reflect a steady-state equilibrium in which the rate of generation is equal to the rate of repair. Thus, the presence of DNA adducts in cells indicates the incompleteness of DNA repair, and these residual lesions have the potential to cause somatic mutations.

Target Genes

The documentation that inherited mutations in specific genes are associated with malignancy suggests that these same genes may be mutated in somatic cells and cause malignancies. Furthermore, specific polymorphisms in these genes could be associated with increased incidence of tumors. The fact that individuals with certain polymorphisms may exhibit increased incidence of certain cancers does not argue against random mutations. The technology for detection of mutations at specific sites in genes in tumors, and for the detection of polymorphisms in human populations, is rapidly becoming available. We should soon have information on the presence of mutations in many genes in cancer cells, at least when they occur at a specific location in the majority of cells within a tumor. However, methods for DNA sequencing are still not yet sufficiently robust to detect random mutations.

DNA Repair Genes. There are four major generic pathways for the repair of DNA lesions in human cells: nucleotide excision repair, base excision repair, mismatch repair, and the direct reversal of lesions (62). Recent studies have increasingly stressed the overlap and redundancy of these pathways (63); specific chemical alterations in
DNA can be repaired by more than one mechanism. Historically, a rare human inherited disease, xeroderma pigmentosa, provided strong evidence linking human mutation in DNA repair genes with cancer (7). Patients with xeroderma pigmentosa are exceptionally prone to skin cancer after exposure to sunlight, and cultured cells from these patients are defective in repair of UV-induced DNA damage. These patients inherit mutations in one of the genes involved in nucleotide excision repair. Mutations in a least one of four mismatch repair genes (hMsh2, hMSH6, hMLH1, and hMS2) are associated with increased incidence of HNPCC and associated tumors. This association between mutations in mismatch repair genes and malignancies has also been demonstrated in mice (reviewed in Ref. 64). We lack evidence that mutations in base excision repair genes are associated with any inherited predisposition to cancer.

**DNA Polymerases.** Until recently, our repertoire of eukaryotic DNA polymerases was limited to five well-characterized enzymes: Pol-α, Pol-δ, and Pol-ɛ, each involved in DNA replication; Pol-β, associated with base excision repair synthesis; and Pol-γ, responsible for mitochondrial DNA synthesis. Pol-β activity is elevated in many cancers, and there are scattered reports on mutations in human colon (65), prostate (66), and bladder (67) cancers. Pol-δ is probably the main replicative DNA polymerase in eukaryotic cells; it has an integral 3′→5′ exonuclease activity that corrects the misincorporation of noncomplementary nucleotides during DNA synthesis. Goldsby and Preston created mice harboring a point mutation in the exonucleolytic domain of Pol-δ that in yeast results in a mutator phenotype. Twenty-three of 48 homozygous mutant mice developed tumors (14 lymphomas, 6 squamous cell carcinomas, and 3 other tumors); no tumors were detected in control or heterozygous animals. These results demonstrate that the production of a mutator phenotype by mutations in DNA polymerases increases the incidence of a variety of malignancies in mice. They provide strong evidence that a mutator phenotype can result in cancer but do not necessarily indicate that most cancers result from a mutator phenotype.

In bacteria, 90% of mutations are dependent on the SOS response that mediates the induction of multiple genes and is associated with mutagenic bypass of DNA lesions. Recently, one of these genes, UmuD’-C (E. coli Pol V), has been shown to encode an error-prone DNA polymerase (68). Simultaneously, a variant of xeroderma pigmentosum (XP-V), which exhibits an elevated mutation rate in cell culture, was demonstrated to harbor a mutation in related DNA polymerases, Pol-η 10, allowing yet another related polymerase, probably Pol-ɛ 10, to bypass UV dimers by incorporating incorrect nucleotides (69, 70). Thus far, some six to eight newly discovered DNA polymerases have been established that could be involved in error-prone bypass of lesions in DNA (71). Studies are under way to determine the expression of these potentially mutagenic enzymes in human tumors.

**DNA Helicases.** Eukaryotic cells contain a surprisingly large number of enzymes that unwind double-stranded DNA prior to being copied by DNA polymerases. These helicases are potential targets for a mutator phenotype. Inherited mutations in several of these DNA helicases are associated with diseases that exhibit a high incidence of cancer. Recently, the genes mutated in Bloom’s syndrome (72) and Werner’s syndrome (73) have been established as encoding DNA helicases belonging to the E. coli RecQ family. Cells with mutations in these genes exhibit characteristic genetic instabilities (74), sister chromosome exchanges in Bloom’s syndrome (75), and large deletions in Werner’s syndrome (76) and thus provide further evidence for the association of genetic instability with cancer. These are uncommon autosomal recessive disorders, and it is important to determine whether the more frequent heterozygotes in the population exhibit an increased incidence of the tumors and if sporadic tumors contain somatic mutations in these helicases.

**Other Target Genes.** There are many genes involved in insuring the stability of the genome. Considering the multiple steps involved in DNA replication, deoxyribonucleotide metabolism, checkpoints in the mitotic cycle, chromosomal segregation, and mitosis, it is apparent that there are many target genes that, if mutated, could induce a mutator phenotype. DNA synthesizing complexes have been isolated from human breast cancer cells that misincorporate nucleotides more frequently than similar complexes isolated in parallel from normal cells (77). The error-prone proteins in these complexes need to be established. Mutations in a number of genes are strongly associated with human cancers, and many of these genes are involved directly or indirectly in DNA transactions. For example, half of all cancers contain mutations in p53 (78). Although there is no strong evidence that mutations in p53 increase point mutations throughout the genome, there is considerable evidence that p53 is involved in DNA repair, apoptosis, and recombination (79, 80), and thus p53 could be a target gene for a mutator phenotype.

**Coupling of Mutation Induction and Clonal Selection**

Although it was generally recognized that both enhanced mutagenesis and clonal selection contribute to a mutator phenotype, it is generally not appreciated that these mechanisms act in synergy. Mao et al. demonstrated that sequential rounds of selection for the growth of E. coli in different restricted conditions resulted in a 5000-fold increase in the rate of spontaneous mutagenesis. If the bacteria were first exposed to a mutagen, the same protocol yielded a population that was 100% mutators. These findings may be particularly relevant to tumor progression (82). As cancers expand, there is a series of host-mediated restrictions that must be overcome; these include reduced oxygen, the requirement for the production of growth and angiogenic factors, and confinement by adjacent tissues (24, 83). Each of these restrictions can be overcome by the selection of mutations in specific genes. With each round of selection there is a “piggy-backing” of mutations in genetic stability genes, and as a result there is an increase in mutation frequencies throughout the genome. Thus, as one selects for mutants, one simultaneously selects for mutators that cause these mutants. With multiple rounds of selection, there is a progressive enrichment of mutations in genetic stability genes.

**Consequences of a Mutator Phenotype**

The hypothesis that cancers express a mutator phenotype and that this phenotype drives the proliferation of cancers and generates variants for clonal selection (Fig. 1) have both theoretical and practical consequences. Among the theoretical implications are:

**Tumor Progression Is Genetically Irreversible**

The concept of a mutator phenotype implies that a large number of mutations are produced randomly throughout the genome of cancer cells; only a few of which result in clonal proliferation. This heterogeneity of mutated genes militates against any possibility of reversion cancer cells to wild-type normal cells. Suppression of these mutations can occur by other mutations or epigenetic silencing and therefore reduce the expression of the cancer phenotype, but this would not re-establish the normal genome. Thus, the classical experiments in

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which tumor cells reverted to normal cells (84) may be at the level of their phenotype but not their genotype.

One cannot eliminate the possibility that epigenetic mechanisms drive tumor progression. In particular, the silencing of DNA repair may facilitate the production of the multiple mutations found in tumors. Silencing of gene expression may be a requirement for cancer cells to tolerate the introduction of aneuploidy. Although strong arguments can be invoked for the role of epigenetic mechanisms in carcinogenesis, we still lack sufficient knowledge of these mechanisms to design critical experiments to validate these concepts.

**The Types of DNA Damage and Mutations Found in a Tumor Are Unlikely to Yield Clues about Initiating Events in Carcinogenesis**

For solid tumors, it takes 20 years or more from the time of exposure to a chemical carcinogen until the clinical detection of a tumor. Thus, it seems improbable that DNA lesions initiating the carcinogenic process would still be present in tumor cells many generations later, unless ongoing DNA damage by the same agent is required for tumor formation. Moreover, the mutations found in a tumor may not result from the most frequent chemical lesions in DNA. Most DNA-damaging agents cause a variety of chemical alterations in DNA; the most frequent lesions may not be the most mutagenic.

Despite the above argument, there are reports that exposure to a specific agent produces a specific mutation. This could indicate that repetitive exposure to a specific agent could be the driving force in clonal selection as might occur in UV-induced skin cancers (85), aflatoxin-induced liver cancers (86), and perhaps smoking-associated lung cancers (87). Exposure to UV induces skin cancers, and the mutations detected in the p53 gene are found opposite pyrimidine dimers and involved T→C or CC→TT tandem substitutions that are characteristic of UV damage (88). Areas with aflatoxin contamination have increased incidence of primary hepatomas, and p53 mutations occur predominately at codon 249 and involve G→T substitutions. A less compelling case is provided by the G→A transversions found in the p53 gene in lung cancers (87). Although a similar substitution is found upon exposure of human epithelial cells to benzo[a]pyrene in tissue culture, G→A transitions are also the result of errors by DNA polymerases. The major question is whether these mutations are diagnostic of specific toxins produced by the carcinogens, or do they reflect selection of mutations in a random population that promote clonal proliferation?

**Benign Cancers Contain Multiple Mutations**

The arguments suggesting that the acquisition of a mutator phenotype is an early event in carcinogenesis imply that early tumors such as colon adenomas and uterine hyperplasia and leiomyomas should contain microsatellite and chromosomal aberrations. Evidence supports this assertion (20, 89). With new techniques it should soon be possible to recreate the archeology of mutated genes that promote clonal expansions as tumors progress.

**Late in Tumorigenesis, Selection May Be against a Mutator Phenotype**

A mutator phenotype may be an early event that drives tumor progression, yet it may no longer be present by the time a tumor is clinically apparent. Although many mutations in cancer cells are neutral or advantageous, some will be deleterious. Thus, there is likely to be a critical threshold, after which selection will be against a mutator phenotype. As a result, tumors may no longer exhibit a mutator phenotype but will never lose the mutator state in their random mutations throughout their genomes.

**Tumors Contain Cells with Preexisting Drug-resistant Mutations**

If one considers the likelihood that a mutator phenotype is an early event in carcinogenesis and the 20 years it takes for a cancer to become clinically apparent, it seems probable that each tumor, consisting of millions of cells, contains one or more cells that have already accumulated mutations rendering them drug or antibody resistant (Fig. 1). The preexistence of these mutations was first demonstrated by Tlsty et al. (90); tumor cell lines, but not normal cell lines, rapidly amplified genes, rendering them resistant to chemotherapeutic agents. Similarly, it is unlikely that there will be antigens that occur in all tumors of a given type and render these tumors susceptible to immunotherapy. The current success of immunotherapy is primarily based on overexpression of normal antigens by specific tumors. Instead, the concept of a mutator phenotype suggests that immunity may have to be tailor made to individual tumors.

**Practical Implications of a Mutator Phenotype in Cancer**

Among the practical implications of a mutator phenotype in cancer are:

**Mutations Are a Marker for Cancer**

Although no specific mutation is absolutely diagnostic of a specific malignancy, the presence of an increased number of mutations may indicate that cells are already on the path to produce a cancer. Both microsatellite instability and chromosomal alterations are being investigated as tumor markers in cells in blood, gastric, and bronchial lavages and pancreatic brushings. In addition, naked DNA is found in serum, and mutations in this DNA can serve as a marker for tumors at distant sites (91, 92). With the development of more sensitive and quantitative techniques, the detection of genetic alterations in cells or DNA, it may be feasible to identify individuals likely to develop specific cancers.

**Emerging Technologies Should Allow One to Quantitate Random Mutations**

Considering that even if the mutation rate of cancer cells are elevated 100-fold early in carcinogenesis, one would still only anticipate one nucleotide alteration per million nucleotides sequenced. This stretches the current level of technology; at best, one may be able to establish nucleotide sequence changes in a very limited number of tumors. Improvements in methodologies by 10–100-fold, coupled with cloning single copies of a gene, should make it possible to quantitate random mutations in multiple tumors and to assess whether mutation accumulation is prognostic for susceptibility to cancer.

**Cancers Arise in a Field of Normal Cells Harboring Multiple Mutations**

Recent studies suggest that colon cancers arise amid a field of premalignant cells that express a mutator phenotype. The studies of Rabinovitch et al. (34) on chromosomal changes in chronic ulcerative colitis provide a persuasive demonstration of this concept. They detected an increased loss of chromosomal arms in cells in rectal biopsies from patients with ulcerative colitis and with associated cancers elsewhere in the colon but not in cells from patients with...
The mutated mucosal cells are at sites distant from the cancer. The implication is that the cancers arose within an extensive field of cells expressing a mutator phenotype. This study is of practical importance because it may provide a simple procedure for the screening of patients with ulcerative colitis who are likely to develop colon cancer.

The presence of random point mutations in precancerous tissue has been reported in both hemochromatosis and Wilson’s disease (93). Both diseases are characterized by an increased incidence of primary hepatoma. Random (nonclonal) mutations in p53 similar to those produced by oxygen-reactive species were detected in nontumorous liver in patients with Wilson’s disease. In the latter study, the assay used provided a uniquely high sensitivity in detecting one mutation in $10^7$ nucleotides and thus may offer an advantage in examining other genes for random events. Using the same approach, Hussain et al. (94) detected p53 mutated alleles in nontumorous colon tissue from patients with ulcerative colitis, further supporting that cancers may arise in a field of mutated cells. Although these studies are limited to precancerous diseases associated with inflammation, they provide additional evidence that mutations in normal tissues precede the onset of detectable malignancies.

Other evidence supports the concept that regional clonal expansion of phenotypically normal but genetically altered cells may precede the development of cancers. The inactivation of an insulin-like growth factor by deletion of a polydeoxyguanosine tract occurs in liver tissue adjacent to hepatocellular carcinomas (95). Regions of loss of heterozygosity have been reported in morphological normal lobes adjacent to breast carcinomas, and clonal areas harboring p53 mutations are found in normal skin. It may be possible that tumors arise in fields of cells that exhibit a higher mutation rate attributable to epigenetic modification. Silber et al. (96) reported that gliomas are surrounded by normal cells that are defective in the repair of DNA damage by alkylating agents (merf), implying lack of $O^6$-alkylguanine-DNA transferase production is a predisposing factor for the development of brain tumors.

**Chemotherapies Directed against Specific Targets or Oncogenes Are Unlikely to Kill All Cancer Cells**

The fact that no single oncogene or suppressor gene is universally mutated or down-regulated in all cases of solid human cancer reinforces the concept that tumors are heterogeneous. The marked heterogeneity of cell populations within a tumor presents a plethora of randomly mutated cells, some of which are likely to contain resistant mutations to chemotherapeutic agents. In the presence of chemotherapy, the mutant tumor cells will have a selective growth advantage and repopulate the tumor. It seems likely that tumors would also contain cells that are resistant to antibodies directed against specific oncogenes. Thus, specific chemotherapies are unlikely to eradicate 100% of cancer cells within a tumor, and one has to rely on host immunological mechanisms, which in many cases are diminished by cancer chemotherapy.
Effects of Increased Mutagenesis

The large number of mutations in a tumor suggests the possibility that the fidelity of DNA replication operates near the error threshold for cell viability. Further errors in DNA replication may be lethal. In support of this concept is the high level of apoptosis in tumors. Furthermore, many drugs used in the treatment of cancer are powerful mutagens, and enhanced mutagenesis may be a significant factor in their therapeutic efficacy. Thus, one should consider the testing of mutagenic nucleoside analogues in the treatment of human malignancies, weighing heavily the problem of inducing second tumors. Of particular utility would be analogues that are not subject to excision by human repair enzymes.

Prevention of Cancer by Delay

If a mutator phenotype drives the carcinogenic process, it might be possible to prevent cancer by delay. A 2-fold reduction in mutation rates could prolong the time between initiation and clinical manifestations of cancer from 20 or more years to 40 or more years. Prevention by delay may be particularly applicable to cancers associated with prolonged chronic inflammation by either bacteria (gastric cancer; Ref. 97), viral (primary hepatitis; Ref. 98, 99), immunological insufficiencies (ulcerative colitis; Ref. 100), or unknown etiology (chronic pancreatitis; Ref. 101). In each disease, the chronic inflammatory response is associated with increased generation of oxygen-reactive species by inflammatory cells. These reactive oxidants are generated by phagocytes to kill bacteria but may also damage host cell DNA. The elimination of the causative agent or the reduction of the inflammatory response would be predicted to delay the clinical appearance of the associated tumors.

Summary

This prospectus on a mutator phenotype suggests that cancer in the adult results from the accumulation of large numbers of somatic mutations. Mutations occur randomly throughout the genome; among these are mutations in genes that function in normal cells to maintain the stability of the genome and to guarantee that it is faithfully copied and transmitted to progeny during each cell division. Mutations in genetic stability genes lead to further mutation throughout the genome, including other genes involved in maintaining genetic stability. Our emphasis has been on DNA polymerases and enzymes involved in DNA repair, but there are many other potential targets for the generation of a mutator phenotype. Processes that lead to the further accumulation of mutations include selection for cells harboring mutator mutants and the constant bombardment of DNA by reactive chemicals produced by normal metabolic processes.

Current advances in DNA sequencing and related technologies should now make it feasible to measure the accumulation of random mutations in human cancer and thus to substantiate the hypothesis of a mutator phenotype in human cancer. It will now be important to determine the distribution of mutant genes in individual cells within a tumor both to trace the evolution of clonal lineages and to identify genes that are mutated in most cells. Among these deep mutations should be those that confer a mutator phenotype. The ensemble of mutated genes within a tumor may define the probability of developing drug resistance or guide drug therapy. Sensitive methods for the detection of random mutations should allow the monitoring of blood and human materials for precancerous cells, which could enable early therapeutic intervention. The most important verification of the mutator phenotype hypothesis may be the demonstration that a reduction in the multiplicity of random mutations in precancerous cells prevents the development of malignancies.

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References


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A Mutator Phenotype in Cancer

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