Multiple mutations and cancer

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Most human tumors are highly heterogenous. We have hypothesized that this heterogeneity results from a mutator phenotype. Our premise is that normal mutation rates are insufficient to account for the multiple mutations found in human cancers, and, instead, that cancers must exhibit a mutator phenotype early during their evolution. Here, we examine the current status and implications of the mutator phenotype hypothesis for the prognosis, treatment, and prevention of human cancers.

Cancer is a disease that develops slowly. For most solid human tumors, there is a 20-year interval from carcinogen exposure to clinical detection. During this time, cancer cells acquire the capacity to divide, invade, and metastasize. The mutator phenotype hypothesis proposes that these phenotypes result from mutations in genes that maintain genetic stability in normal cells. Mutations in genetic stability genes can cause mutations in other genes that govern genetic stability, initiating a cascade of mutations throughout the genome. Some of the resulting mutations will confer a selective advantage(s), allowing the mutated cells to expand and achieve clonal dominance.

In this perspective, we first consider the evidence that individual malignant cells in human tumors contain thousands of random mutations. Extensive heterogeneity of tumor genomes has important implications for cancer treatment. Second, we evaluate the potential roles of a mutator phenotype (1) and selection (2) during tumor progression. Third, because it is instructive, we analyze important evidence countering the mutator phenotype concept, e.g., studies indicating that a defined sequence of specific cancer-associated mutations occurs during tumorigenesis, and other theories suggesting that the large numbers of mutations found in cancers can result from normal replicative processes. Last, we consider the implications of a mutator phenotype for cancer therapy.

The Mutator Phenotype Hypothesis

For many years, my colleagues and I have proposed and developed the hypothesis that cancer cells exhibit a mutator phenotype, e.g., ref. 1. The basic premise is that normal mutation rates are insufficient to account for the multiple mutations observed in cancer cells, and, therefore, mutations that increase mutation rates are essential to account for the large numbers of mutations observed in human tumors. The mutator phenotype hypothesis was originally postulated for mutations in genes that control the fidelity of DNA replication and/or the efficacy of DNA repair. We now know that mutator mutations can arise in genes whose products function in additional processes that maintain the normal DNA sequence in cells. Hence, this hypothesis has evolved to encompass more recently discovered genes (3) that govern processes such as chromosome segregation, damage surveillance (e.g., checkpoint control) and cellular responses (e.g., apoptosis). The mutator phenotype arising from mutations in genetic stability genes can have diverse manifestations, such as point mutations, microsatellite instability, and loss of heterozygosity (LOH).

Evidence for Multiple Mutations in Human Cancers

Mutations are heritable changes in the nucleotide sequence of DNA and, as such, include chromosomal abnormalities. Until recently, evidence for multiple mutations in human cancers was based mainly on chromosome aberrations. Although multiple abnormal chromosomes are found in most solid tumors, they are seldom diagnostic of tumor type nor are they indicative of prognosis. An exception is the characteristic translocations involving rearrangements of specific chromosomes that have been instrumental in the diagnosis of several hematologic malignancies and sarcomas. Comparative genomic hybridization (CGH), a technique that measures changes in DNA copy number, and spectral karyotyping (SKY), the visualization of chromosome rearrangements, have afforded higher resolution than cytological observations, but detection of mutations is still limited to stretches spanning millions of nucleotides. In most tumors, there are multiple regions that exhibit abnormalities in CGH (4) and SKY (5). Augmenting this evidence are the demonstrations that tumors display amplification of segments of DNA at high frequencies (6) and also exhibit loss of heterozygosity LOH resulting from deletions in one of the parental chromosomes (7). Importantly, Klein *et al.* (8), by applying both CGH and LOH to single metastatic cells in human bone marrow, demonstrated multiple chromosome alterations in single cancer cells. CGS and LOH survey only small fractions of the genome; extrapolation to the entire genome suggests that tumors contain thousands of mutations (9).

Direct evidence for thousands of mutations in cancer cells has come from the observations of changes in the length of repetitive nucleotide tracts (microsatellites) in tumor DNA from patients with hereditary nonpolyposis coli (HNPCC). These patients harbor mutations in mismatch repair genes (10, 11). Failure of the mismatch repair system to correct slippage errors made by DNA polymerases during copying of repetitive sequences gives rise to the length changes (microsatellite instability). It is estimated that as many as 100,000 repetitive sequences per genome are altered in HNPCC. Investigation of sporadic colon cancers also indicates that a large number of additions and deletions have occurred in sequences between repetitive elements (12), some of which could result from reduced expression of mismatch repair genes (13). In addition, microsatellite instability has been detected in a variety of tumors that are not known to harbor mutations in mismatch repair genes, and in premalignant conditions associated with chronic inflammation (12), suggesting that changes in cellular environments can induce a transient deficiency in mismatch repair and result in a mutator phenotype. Because repetitive sequences are also present within exons, length instability can be manifested as frameshift mutations that inactivate genes, including genes that suppress tumor formation. Importantly, repetitive sequences have been found within the coding regions of several genomic stability and growth regulatory genes, including hMSH3, hMSH6, TGF-β, APC, IGF-RII, and BAX (listed in ref. 14), and somatic cells exhibiting increased microsatellite instability also exhibit increased mutation rates in expressed genes (15). Thus, it seems likely that repetitive sequences are "hot spots" for mutagenesis and may serve as markers for the presence of other types of mutations throughout the genome.

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Maintenance of the Genome

The genome of normal cells is in a dynamic equilibrium, representing a balance between processes that generate mutations and processes that maintain the normal nucleotide sequence. In the forward direction, large numbers of reactive cellular metabolites and environmental agents alter the chemical structure of DNA, and these alterations can result in mutations upon subsequent replication; moreover, DNA polymerases generate mutations even when copying undamaged DNA. In the reverse direction, a multiplicity of DNA repair processes restore the normal nucleotide sequence. The initial hypothesis of a mutator phenotype was focused on mutations that diminish the fidelity of DNA synthesis. In normal cells, duplication of the base sequence in nuclear DNA is exceptionally accurate, the overall error rate being one misincorporated base for every billion nucleotides polymerized. Contributions to this accuracy include the free energy differences between complementary and noncomplementary base pairs (a factor of 10^2), nucleotide discrimination at the polymerase active site (10^3 to 10^5), excision of noncomplementary nucleotides immediately after incorporation (10^2 to 10^3), and postsynthetic mismatch correction (10^3) (16). Together these processes can account for the accuracy of DNA replication. DNA polymerase-δ (pol-δ) appears to be the major DNA polymerase that catalyzes eukaryotic DNA replication. Replacement of a wild-type DNA pol-δ gene with a mutated allele that lacks proofreading activity results in both lymphomas and epithelial cancers in mice (17), indicating that reduction in polymerase accuracy by abolishment of proofreading is sufficient to increase cancer incidence. Considering the involvement of DNA polymerases in DNA replicative processes, it is difficult to envision that inherited mutations in DNA polymerases that dramatically alter function are compatible with normal human development. Nevertheless, such mutations could arise in somatic cells and generate tumors. In the case of *Escherichia coli* DNA polymerase I, a combination of mutations that separately abolish proofreading and reduce base selection can result in a 10,000-fold increase in misincorporation (M. Camps and L.A.L., unpublished data). Also, in yeast, there is a synergism between polymerase mutations and mutations in mismatch correction (18). Thus, multiple mutations in mechanisms that guarantee the fidelity of DNA replication could have profound effects on mutation rates and carcinogenesis.

Damage to DNA in human cells is continuous and very extensive. For example, there is evidence that DNA damage by reactive oxygen species generates 10,000 residues of 8-oxo-deoxyguanosine per cell per day, and this is just one of the many normal reactive molecules that damage cellular DNA (19). The accumulation of DNA damage may be of particular importance in stem cells that persist for long periods of time and have the potential to differentiate into a variety of tissues; in fact, there is considerable evidence that cancers arise in stem cells or early progenitor cells. To counteract this damage, cells have evolved elaborate processes for detecting and repairing damaged DNA and/or preventing altered chromosomes from passing to daughter cells. Rare inherited human diseases caused by mutations in DNA repair genes are associated, with few exceptions, with markedly increased incidences of specific cancers.

Many cancers arise in a background of chronic inflammation. The inflammatory process could drive carcinogenesis by generating reactive oxygen species and stimulating reparative proliferative processes in the inflamed tissue. For example, in chronic ulcerative colitis, adenocarcinomas arise in a field of chronically inflamed colonic epithelial cells that harbor multiple chromosome alterations and display dysplastic features (20). Other inflammatory processes that are tightly associated with the induction of malignancies at the site of inflammation include pancreatitis, hepatitis, and chronic infection of the gastric mucosa with *Helicobacter pylori*. Infection with *H. pylori* is also causally linked with the onset of a specific type of lymphoma; early during tumor progression, these MALT lymphomas regress in response to antibiotic therapy that targets the *H. pylori* infection (21). Later, the tumors lose responsiveness, suggesting that they have accumulated genetic abnormalities that enable autonomous growth in the absence of stimulation by infection.

Mutations resulting from unrepaired DNA damage in normal human cells are also prevented by checkpoint pathways. The activation of checkpoint pathways results in either cell cycle arrest, extending the time available for DNA repair, or alternatively, can trigger apoptosis when DNA damage is too extensive. The net result is to limit the amount of DNA damage and/or mutations inherited by daughter cells. It has been estimated that at least 50% of human tumors are defective in a checkpoint pathway involving p53, and many other tumors are defective in interacting pathways involving genes encoding the checkpoint proteins p16 and p19ARF (22). Mice harboring deletion of these genes exhibit an increased incidence of spontaneous tumors. In follicular lymphoma, BCL-2 is overexpressed, the cells fail to undergo apoptosis, and, as a result, progress to more malignant lymphomas (23). It should be noted that tumors defective for a particular checkpoint pathway may be more sensitive to agents that produce damage normally detected by that checkpoint. Elaborate genetic screens in yeast have been established to identify drugs that selectively target cells with mutations in specific checkpoint pathways.

Types of Genetic Instability

It has been proposed that genetic instability in human cancers can be divided into two types: microsatellite instability (MIN), which is usually equated with DNA polymerase errors, and chromosomal instability (CIN), which can result from errors in chromosome partitioning (24). In the case of colorectal and endometrial cancers, most tumors exhibit either CIN or MIN, but not both. This dichotomy is consistent with the response of cells in culture to different chemical carcinogens (25). It is important to note that CIN and MIN are well characterized as a result of facile detection by current methods; MIN is detected by PCR-based analysis, and CIN is detected by karyotype analysis. In contrast, detection of random mutations, particularly point mutations, is more difficult (see below), and this difficulty has limited the search for evidence of a mutator phenotype at the single nucleotide level. Thus, it seems possible that in any given tumor, MIN or CIN may be secondary to an undetected primary instability manifested as random point mutations throughout the genome. This instability could result from mutations in DNA polymerases or other genes that determine the fidelity of DNA replication or other DNA synthetic processes. In the case of DNA polymerases, the most potent mutations would be those that decrease base discrimination but do not compromise catalytic efficiency. We have recently found that many DNA polymerase mutants harboring mutations at the active site exhibit these characteristics (26).

With respect to the postulate of a mutator phenotype at the single nucleotide level, it is expected that the mutations generated would be located throughout the genome. Viewed globally, these mutations would be distributed randomly, even though there would be "hot spots" and "cold spots" governed by the structure of DNA, the presence of repetitive sequences, and bound proteins that shield or sensitize DNA to reactive molecules. The essentially random distribution of mutations renders current methods inadequate for their detection; DNA sequencing, the gold standard, identifies only the most frequent nucleotide substitutions at each position. As illustrated in Fig. 1, only substitutions at each position that are present in at least 10% of DNA molecules would be identified; such mutations pre-

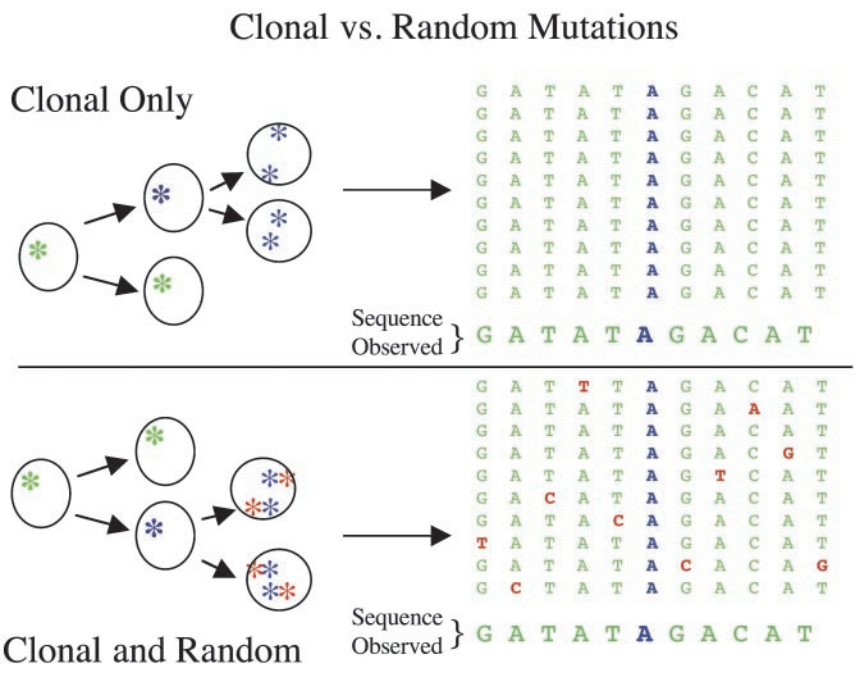

Fig. 1. A mutator phenotype arising from genetic instability at the single nucleotide level is not detected in routine DNA sequencing. The DNA sequences of oligonucleotides containing clonal and random mutations are shown. Clonal nucleotide substitutions are in blue, and random substitutions are shown in red. The only substitutions observed by DNA sequencing are those present in the majority of molecules, i.e., the clonal mutations. In this example, random substitutions that constitute <10% of the nucleotides at each position are not detected. This level of detection approximates the sensitivity of routine DNA sequencing.

sumably result from clonal proliferation. Thus, extensive microheterogeneity within a population of molecules would not be detected by DNA sequencing. In fact, in the example in Fig. 1, the observed sequence is not found in any of the individual oligonucleotides, each of which contains a random substitution. To detect random base substitutions that do not lead to clonal proliferation, it is necessary to start with single cells or to sequence single DNA molecules. Thus, the number of random point mutations present in tumors, and the prevalence of a mutator phenotype at the single nucleotide level, are issues awaiting definitive resolution.

Analysis suggests that a limited number of rate-determining events occur in tumorigenesis. Knudsen's model for the generation of retinoblastoma is based on hits in both alleles of the same gene (27). The exponential increase in adult tumors as a function of age indicates that there are four to seven rate-determining events that could be mutations in critical genes. Transformation of rodent cells in culture indicates that at least two genetic changes occur before cells acquire the ability to form tumors, and additional genetic changes are required for malignant transformation of human cells in culture (28). Weinberg and colleagues have hypothesized that there are at least six required capabilities for tumor formation. Considering that DNA damaging agents do not target specific genes, it seems likely that vast numbers of mutations are generated early during carcinogenesis, and that there is selection among these for those mutations that are rate-limiting for tumor formation (29).

Mutations in Genes That Control Genetic Stability

The mutator phenotype hypothesis postulates that an initial mutator mutation generates further mutations, including mutations in additional genetic stability genes, resulting in a cascade of mutations throughout the genome (30). The initial mutation that starts the cascade presumably arises from rare stochastic events. Such events could involve DNA damage generated either by cellular processes or by exogenous mutagens. For example, a failure of DNA repair to remove a lesion in front of an advancing replication fork could lead to incorporation of a noncomplementary nucleotide during DNA replication. Alternatively, a DNA synthesis error resulting from the low, intrinsic infidelity of a major replicative DNA polymerase copying undamaged DNA might escape repair. Or, for example, a synthesis error might arise from the action of one of numerous, recently identified error-prone DNA polymerases (31, 32). The first mutator mutation could occur in any of the numerous genes controlling processes that ensure genomic integrity. The multiplicity of DNA polymerases and associated proteins in cells, and of DNA repair pathways, provides numerous target genes that, when mutated, can result in genetic instability and a mutator phenotype at the level of point mutations (1).

A mutator phenotype would need to be expressed early in tumorigenesis to generate the causally associated mutations that drive tumor progression. However, it may be difficult to document elevated mutation rates underlying a mutator phenotype that occurs early in tumor formation. First, tumors may arise in fields of cytologically normal-appearing cells that already contain multiple genetic changes. The idea of a field of premalignant cells in which tumors arise is supported by studies on chronic infectious diseases that exhibit a high incidence of cancers at the site of infection. Second, multiple chromosome alterations have been demonstrated in benign tumors (33); these tumors are encapsulated, fail to invade or metastasize, and thus may be derived from clones that occurred early during tumorigenesis but fail to acquire the mutations required for malignant transformation. Third, during the later stages of progression, when tumors are well established, accumulation of further mutations might become disadvantageous, and selection may discriminate against cells with elevated mutation rates. Evidence for this idea can be inferred from the large numbers of apoptotic cells and abnormal mitotic figures present in high-grade tumors. However, even if mutator alleles are lost from tumors, and mutation rates return to normal, cancer cells should retain the signature of a mutator phenotype: multiple mutations distributed throughout the genome. In cases where a mutator phenotype at the single nucleotide level is involved, noncoding mutations should be particularly prevalent because they are unlikely to be subject to selection.

It is important to recognize that a mutator phenotype can result from mechanisms other than mutations in critical genes. Profound effects on the evolution of tumor cells, including increased mutagenesis, can be caused by processes such as aberrant gene expression (34) or DNA methylation (35). Even the accuracy of DNA synthesis can be altered by gene expression. For example, in bacteria, mutagenesis depends on the SOS response (36), which involves the induction of an error-prone DNA polymerase that copies past unrepaired bulky alterations in DNA (37). Similar error-prone bypass DNA polymerases have been documented in human cells, and these may have a central role in mutagenesis. In the variant form of Xeroderma pigmentosum (XP-V), a mutation is found in DNA polymerase-η that copies past UV-damage by incorpo-

rating complementary nucleotides. In the absence of DNA polymerase-η, DNA polymerase-ζ catalyzes error-prone replication opposite the UV-damage-induced pyrimidine dimers (38). It remains to be determined whether error-prone DNA polymerases are induced during tumor evolution and result in a mutator phenotype.

Clonal Selection as an Alternative Mechanism for the Generation of Multiple Mutations

The hypothesis that tumor progression involves successive waves of clonal selection was initially introduced by Nowell (2). Under this hypothesis, each selected mutation imparts a proliferative advantage, resulting in successive clonal lineages. The appearance of sequential chromosome alterations has been demonstrated in malignant melanoma (39), and most extensively in adenocarcinoma of the colon (40). A concern with the idea of sequential mutations followed by stringent selection is that each mutation would have to confer a large selective advantage, even recessive mutations that occur in only one of two alleles. Although a sequential model for mutation accumulation has been proposed for the genesis of human colon cancer (40), only a small fraction of the tumors (6.6%) have been shown to contain the three most frequently delineated mutations (41). These results imply that multiple mutations in addition to those commonly found may be involved in the generation of colon cancers.

A mutator phenotype and selection for mutations in specific oncogenes and tumor supressor genes are not mutually exclusive concepts; in fact, it is now widely accepted that mutations could occur randomly, and mutations that govern clonal proliferation could be selected. Moreover, these processes may be interdependent. Sequential rounds of selection for mutations that enhance proliferation in *E. coli* result in a population of cells that express a mutator phenotype (42). With each round of selection for different advantageous mutations, there is concurrent selection for mutations in genes that produce the mutations in these genes, (i.e., mutator mutants). A similar hitchhiking of mutator genes during sequential rounds of selection has also been demonstrated in yeast (R. Kolodner, personal communication) and might be applicable to the growth of tumors where selection is for mutants that exhibit a growth advantage under different conditions. Hanahan and Weinberg (43), in a comprehensive analysis, have identified six essential alterations in cell physiology that guide malignant growth; each of these changes may require mutations that provide a selective growth advantage and may concomitantly enrich for mutator alleles. Thus, both clonal selection and the generation of mutator mutants may be operative during tumor progression, and the predominant pathway may be different in different tumors.

Arguments Against a Mutator Hypothesis in Cancer

Recently, at least four arguments have been advanced against either a mutator phenotype in cancer or a mutator phenotype at the single nucleotide level. It is instructive to review these arguments in detail.

First, it is frequently contended that, because most mutations are detrimental, tumor cells that accumulate large numbers of mutations would not exhibit a selective growth advantage. However, continuous pressure for robustly growing cells during tumor progression would eliminate detrimental mutations. Moreover, recent studies using random sequence mutagenesis have revealed that at least some proteins exhibit remarkable plasticity, tolerating multiple substitutions even within highly conserved motifs that are required for function (26). For example, a mutant of human O^6-methylguanine–DNA methyltransferase (MGMT, also known as AGT) that harbors eight mutations within the active site is as active as the wild-type protein (44). Lastly, in cocultivation experiments with wild-type and mutator bacteria, it is the mutator that outgrows the wild type. Thus, cancer cells might tolerate a high level of mutagenesis without growth disadvantage.

Second, Duesburg and his associates have proposed that the generation of aneuploidy is the initiating event in the conversion of normal cells to cancer cells (45). They emphasize that aneuploidy is very frequent in human tumors and that aneuploidy can be induced in cell lines by incubation with carcinogenic chemicals. They present evidence that aneuploidy may precede the acquisition of a transformed phenotype in cultured cells. Yet, aneuploidy is seldom found in all malignant cells within any tumor, nor is it invariably present in all tumors. For example, 15% of Ewing's sarcomas exhibit a characteristic translocation and are not aneuploid (46), and in Barrett's esophagus, a premalignant condition, alterations in p53, p16, and increased tetraploidy precede aneuploidy (47). These findings indicate that, even though anueploidy is common in human tumors, it is neither a clonal marker nor an initial event.

Third, Bodmer and his colleagues (48) have presented a mathematical model indicating that an increase in mutation rate is not essential for tumor development, and that clonal selection followed by clonal expansion can account for thousands of mutations in colon cancer cells. They estimate that from birth to adulthood, normal colon stem cells undergo 45 divisions, 5,000 divisions occurring during adulthood and 1,000 more during tumor development. Assuming a rate of 5×10^{-9} mutations per base pair and a nonhierarchical order for stem cell proliferation in colonic crypts, one can account for 150,000 mutations per cell in adenocarcinoma of the colon. Their mathematical model indicates that most of the mutations arise during the 5,000 normal cell divisions. They conclude that an increase in mutation rate in tumors is an epiphenomenon and is not responsible for tumor progression. Although many assumptions in this model differ from those embodied in the mutator phenotype hypothesis, the major difference is the 5,000 division cycles undergone by normal colonic stem cells. Importantly, in tissues other than colon or skin, stem cells are unlikely to undergo that many divisions and thus would not accumulate large numbers of mutations. Although we lack measurements of mutation rates in stem cells, the observed increase in mutation frequencies in human kidney tubule cells during the human life span is at most 5-fold (49). It should be noted that only a few clonal mutations have been found in colon cancer cell lines, and yet these were presumably derived from stem cells that would have undergone thousands of cell divisions before malignant transformation and thus should contain large numbers of mutations (see below). Thus, extensive division of stem cells is unlikely to be a universal finding in most tissues.

Last, a recent study has described the sequencing of 3.2 megabases of exonic DNA from 12 sporadic colon cancer cell lines (50). A total of 320 substitutions in coding nucleotides were detected; 90 were previously identified single-nucleotide polymorphisms (SNPs) and 227 were present in DNA from normal cells from the corresponding patients. Only three new tumor-specific coding mutations were identified. Assuming that this mutation frequency prevails throughout the genome, there would be 3,000 mutations that code for different amino acid substitutions in the tumor DNA that are not present in DNA in normal cells from the same individual. Of interest would be data on the number of noncoding mutations that accumulated in these same sequences; one would expect an equal or perhaps greater number, because many coding mutations might have been disadvantageous and hence eliminated during tumor growth. In any case, the authors conclude that, because cells lining the intestine are replaced every few days, the number of base substitutions observed could result from normal mutation rates;

hence, they argue that sporadic colorectal cancer cells need not exhibit a mutator phenotype with respect to point mutations. Instead, these authors postulate that sporadic colorectal cancer cells exhibit a mutator phenotype involving mutations in genes that govern chromosomal stability. We should note that random base substitutions would have gone undetected in this work, because the mutation analysis used multiple copies of DNA that were derived from many cells and were not cloned (see above and Fig. 1). Thus, although this work provides important information on the number of clonal mutations in cancer cells, it does not quantitate the number of random mutations within a tumor.

Implications of a Mutator Phenotype for Cancer Therapy

It remains to be determined whether the acquisition of a mutator phenotype underlies tumor progression and when the enhancement in mutation rate occurs during the life of tumors. In order for a mutator phenotype to be necessary for tumor progression, it would have to occur early and result in the accumulation of large numbers of random mutations. The existence of multiple mutations in cancer cells has important implications.

Stratification. The presence of large numbers of random mutations in a tumor cell population provides abundant, diverse genetic variants for the selection of mutants that specify the cancer phenotype. It may be possible to stratify tumors based on the frequency of random mutations in the genome; tumors with fewer mutations may be less likely to become resistant to chemotherapeutic agents. With early detection it may be feasible to detect minimal tumors that have not accumulated large numbers of mutations and are unlikely to progress; such tumors might be found to have a relatively good prognosis and therapy could be tailored accordingly. Specific types of randomly distributed mutations may serve as footprints for the genetic system that has been compromised, and thus guide treatment to prevent further mutation accumulation.

Tumor Evolution. The evolution of a tumor is monitored by immunologic defenses that recognize tumor antigens. New tumor clones are presumably those that have circumvented the host's immunologic defenses that eliminate mutant cells. Random mutations affecting proteins within cells that have not undergone clonal proliferation may not have tested the host's immunologic defenses and yet may be able to elicit a strong immunologic response. Immunotherapy directed against surface antigens that are unique to tumor cells are unlikely to be effective targets for obliteration of most tumors. Instead, the most effective immunotherapy may require immunization against normal genes that are overexpressed in tumors or immunization against the individual's own tumor that expresses a variety of mutant proteins.

Treatment. The presence of thousands of mutations in single cancer cells suggests that among the 10^8 cells in a human tumor at the time of diagnosis there are billions of different mutations, and that mutations in most, if not every, gene and regulatory sequence are present in one or more cells within a tumor. On exposure to a chemotherapeutic agent, tumor cells with preexisting mutations that render them resistant would preferentially proliferate and could eventually repopulate the tumor, thus accounting for the efficiency with which tumors acquire drug resistance. The efficacy of combination chemotherapy is usually rationalized on the basis of reduced toxicities, each agent being given in subtoxic amounts; killing of tumor cells is additive, but toxicity is not. The presence of random mutations within a tumor provides another rationalization to account for the utility of combined chemotherapy. Combined regimens may elicit a therapeutic response, even in the presence of preexisting mutations that defeat single-agent therapy, because resistance would require that the same tumor cell harbor mutations that confer resistance to each agent.

Most chemotherapeutic agents target either DNA synthesis or mitosis. Thus, it has been difficult to postulate mechanisms for specificity, because these processes occur with the same frequencies in cancer cells as in some rapidly dividing normal cells. It is interesting to consider that most chemotherapeutic agents are potent mutagens, and presumably induce mutations in both normal and malignant cells. The presence of large numbers of preexisting mutations in tumor cells increases the likelihood that additional mutations might exceed a maximum level consistent with viability. Thus, further mutagenesis might be selectively lethal for tumor cells that contain large numbers of mutations. It seems plausible that there is an error threshold for cell viability, and it is interesting to speculate that some anticancer drugs may introduce a sufficient number of mutations in cancer cells that already harbor multiple mutations to exceed this threshold (51).

New Opportunities. Although the concept that cancer results from a mutator phenotype introduces many impediments for cancer chemotherapy, it also presents new opportunities for cancer prevention. If random mutations are rate-limiting for tumor progression, it might be feasible to prevent the clinical manifestations of a tumor by inhibiting mutation accumulation. Even a 2-fold decrease in mutation accumulation could introduce a significant delay in the clinical manifestations of a tumor (52). If one could double the number of years it takes for cells to accumulate the requisite number of mutations required for invasiveness and/or metastasis, one would significantly reduce the life-threatening manifestations of cancer. Methods to reduce endogenous DNA damage need to be explored and in particular DNA damage by reactive oxygen species. In addition if mutations occur during tumor progression that render DNA polymerases error-prone, they are also likely to enhance their ability to incorporate nucleotide analogs that terminate DNA synthesis or act as suicide agents. Lastly, if error prone DNA polymerases are responsible for mutation accumulation during tumor progression, inhibitors directed against these polymerases might reduce mutation accumulation without inhibiting normal replication (53).

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