Published in final edited form as:

Curr Drug Targets. 2010 October ; 11(10): 1296–1303.

The Mutator Phenotype in Cancer: Molecular Mechanisms and Targeting Strategies

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Abstract

Normal human cells replicate their DNA with exceptional accuracy. It has been estimated that approximately one error occurs during DNA replication for each 10^9 to 10^{10} nucleotides polymerized. In contrast, malignant cells exhibit multiple chromosomal abnormalities and contain tens of thousands of alterations in the nucleotide sequence of nuclear DNA. To account for the disparity between the rarity of mutations in normal cells and the large numbers of mutations present in cancer, we have hypothesized that during tumor development, cancer cells exhibit a mutator phenotype. As a defining feature of cancer, the mutator phenotype remains an as-yet unexplored therapeutic target: by reducing the rate at which mutations accumulate it may be possible to significantly delay tumor development; conversely, the large number of mutations in cancer may make cancer cells more sensitive to cell killing by increasing the mutation rate. Here we summarize the evidence for the mutator phenotype hypothesis in cancer and explore how the increased frequency of random mutations during the evolution of human tumors provides new approaches for the design of cancer chemotherapy.

Keywords

Mutator phenotype; lethal mutagenesis; error catastrophe; cancer genome

INTRODUCTION

Cancer is characterized by mutations that enable unregulated cell division, invasion, and metastasis. We have hypothesized that tens of thousands of genetic alterations in most human cancers do not result from the low inherent mutation rates exhibited by normal human cells; instead, cancer cells express a mutator phenotype [1]. The underlying premise is that mutation rates in normal human cells are insufficient to account for the multiple mutations found in human cancers, and that cancers must therefore exhibit an increased mutation rate early in their evolution. This concept was initially formulated based on

CONFLICT OF INTEREST

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mutations in DNA polymerases that render them error-prone, and mutations in enzymes involved in DNA repair that decrease the ability of cells to remove potentially mutagenic DNA lesions [2]. As a result, there is increased genomic instability with the acquisition of mutations in oncogenes and tumor suppressor genes. With the increased number of genes shown to be required for guaranteeing genetic fidelity, the mutator phenotype hypothesis has been broadened to include any gene involved in the maintenance of genetic stability [3].

Until recently, support for the concept of a mutator phenotype in human cancers has been based primarily on theoretical models. Of particular importance are models that address the likelihood that a tumor would evolve by a mutator pathway. If one to three driver mutations are required, one need not invoke increased mutation rates in cancer cells to account for the emergence of a tumor [4]. However, if a large number of driver mutations are required, mutator mutations will greatly accelerate carcinogenesis, particularly if they are early events. These models also consider impact of increased cell death [5], and of clonal expansion [6]. Recently, methods have been developed to measure the number of random mutations in a tumor, allowing the beginnings of experimental verification of these models [6]. Additional support for a mutator phenotype comes from the sequencing of a variety of human tumors, in which hundreds to thousands of clonal mutations have been described in each tumor that has been analyzed (vide infra).

EXPRESSION OF A MUTATOR PHENOTYPE

In contrast to the rarity of mutations in normal cells, the genomes of cancer cells contain multiple changes. Theodor Boveri first suggested that malignancy resulted from a disturbance of chromosomal balance and, although specific chromosomal alterations are diagnostic of only a few types of cancer, chromosomal aberrations are observed in most tumors [7]. Multiple chromosomal alterations occur in both benign and malignant tumors and include deletions, additions, amplifications, and translocations, frequently involving millions of nucleotides [8]. Studies on DNA copy number and loss of heterozygosity (LOH) have established that individual tumors from a variety of cancer types can contain more than 30 independent clonal chromosomal alterations [9, 10]. Primary adenocarcinomas of the breast (and colon) frequently harbor more than 20 different chromosomal alterations [11, 12], and individual disseminated breast cancer cells have been shown to carry a diverse spectrum of chromosomal abnormalities distinct from that of the primary cancer [13]. These results suggest that chromosomal abnormalities continually accumulate during tumorigenesis even subsequent to the clinical appearance of a tumor.

Definitive evidence for multiple nucleotide sequence changes within the cancer genome came initially from findings in Lynch syndrome (also known as hereditary non-polyposis colorectal cancer (HNPCC) syndrome) and in a subset of sporadic colorectal tumors [14–16]. In hereditary colorectal cancers, which involve mutations in mismatch repair genes, microsatellites and other repetitive sequences throughout the genome display extensive somatic length variations. Based on the frequency of mutations in a limited number of microsatellites examined, it is estimated that thousands of loci are mutated within each tumor cell [17]. Repetitive DNA sequences are mutational "hotspots" in which slippage by DNA polymerases exceeds the capacity for correction by mismatch repair. This results in

elongation or shortening of the repeats, and can potentially cause frame-shift mutations within coding sequences or alter the spacing between DNA regulatory elements [18]. In addition, this mismatch repair mutator phenotype results in a dramatic increase in the frequency of random somatic point mutations [19]. In chronic ulcerative colitis (UC), the presence of microsatellite mutations at sites that are distant from areas of dysplasia provides a powerful marker to foretell the likelihood for the development of malignancy, and offers the potential to map cell lineages [20]. While high levels of microsatellite instability also occur in 10–15% of sporadic colorectal cancer, it has been speculated that length alterations of repetitive sequences are likely present in all tumors, provided enough markers are assayed [21].

The recent effort to characterize the human cancer genome has yielded a wealth of information about clonal mutations present in a variety of human tumors. Complete sequencing of all predicted human coding exons in breast and colon cancer identified medians of 84 and 76 non-silent mutations per tumor, respectively [12, 22]. While almost 10% of the 18,197 genes studied were detectably mutated in at least one specimen, each tumor displayed a unique and highly varied profile of mutated genes. Contrary to expectations, other than the few genes previously known to be frequently mutated in these cancer types (e.g., *TP53* and *APQ*), no new mutated genes were identified that occurred at a high prevalence. Wood *et al.*, thus proposed that the cancer genome is composed of a larger number of infrequently mutated genes, each providing a small fitness advantage, and few commonly mutated genes [12]. They concluded that the wide variation in tumor behavior and responsiveness to therapy is likely due to this clonal mutational heterogeneity.

Follow-up studies on diverse cancer types have now generated extensive catalogs of clonal somatic point mutations in a variety of tumor types (Table 1). The most fundamental conclusion to be drawn from these studies is that all cancer types studied to date display significant heterogeneity at the genetic level, presumably in part as a consequence of their underlying mutator phenotypes [23]. Individual solid organ tumors typically harbor non-silent clonal genetic alterations in more than 50 different genes. Despite the fact that a small number of these genes are mutated in a high proportion of cancers, the prevalences at which the majority are mutated within different tumors of the same cancer type is low. These results are not in accord with the classical paradigm, in which a small number of key genes, mutated in a linear temporal sequence, drive tumorigenesis.

A significant limitation is that these studies focused exclusively on identifying mutations in exons of known protein-coding genes. With the ever-increasing recognition of the importance of non-coding DNA, including regulatory regions and sequences that encode noncoding RNAs, it can be argued that the total number of functionally consequential clonal mutations must be greater than those identified in coding sequences of genes. The first unbiased whole genome sequencing study was performed on an acute myeloid leukemia (AML) genome and constituted the first complete description of all clonal sequence alterations within a single cancer [24]. By exhaustively interrogating the cancer genome and, importantly, the paired normal genome, of a single AML type M1 patient using massively-parallel sequencing, Ley and colleagues identified 500 to 1,000 somatic mutations, of which less than 2 percent were in protein-coding genes. Although the overall number of protein-

coding mutations (10) is fewer than that described for solid organ tumors (Table 1), similar to the pattern observed in other cancer types, none of the 8 newly identified genes were found to be mutated in a further 187 cases of AML. These results have been replicated in a follow-up study confirming that, genome-wide, approximately 750 clonal mutations are present in the minimally cytogenetically altered AML-M1 cancer genome [25]. A similar pattern has also recently emerged for the genomes of solid tumors; unbiased whole genome sequencing has uncovered greater than 20,000 mutations in the cancer genomes of cell lines derived from metastatic melanoma and non-small cell lung cancer, with less than 1% of these being non-synonymous mutations in coding regions [26, 27]. While superficially these findings potentially provide a basis for reconciling the epidemiological data, which suggest that four to seven key mutations are required for tumorigenesis [28], and the pervasive consequences of a mutator phenotype, this likely reflect an under-appreciation of the importance of mutations in non-coding regions of the cancer genome.

Most methods for detecting chromosomal or nucleotide changes score only clonal alterations and exclude changes that could be present in only a small fraction of cancer cells within a tumor. Therefore, though extensive, these data merely represent the tip of an iceberg. The cancer genome is more than simply a sum of its clonal mutations. A primary tumor is itself genomically heterogeneous, with each cell having a unique mutational signature. This genetic variation within a cancer cell population reflects its evolution and, importantly, serves as a reservoir of genetic diversity from which resistance to therapy may arise. Analysis of disseminated single cells in minimal residual disease has shown that there is a high level of genomic heterogeneity within individual lesions as well as between primary tumors and metastatic cells [29]. While some of the current sequencing technologies have the potential to detect subclones with frequencies as low as 1 in 1,000 [30], detecting rarer subclones by DNA sequencing will require the evolution of more accurate thermostable polymerases. This intra-species mutational diversity could be a major contributor to the diverse spectrum of clinical phenotypes.

To identify all the alterations present in an individual tumor, it is necessary to interrogate single DNA molecules. The accuracy of current deep sequencing methods is inadequate to detect random mutations in single-molecules lower than a frequency of 10^{-4} . We have adapted a RFLP/PCR based assay to identify mutations in single molecules that render the DNA non-cleavable by restriction enzymes. Mutations are then quantified by amplification of single molecules with primers flanking the intact cut site using real-time PCR and confirmed by DNA sequencing [31]. This random mutation capture (RMC) assay permits the calculation of random mutation frequency of the cells that compose various tumor and normal tissues. In contrast to the low frequency of mutations in normal human tissues, adjacent tumor tissues were found to exhibit a high frequency of single-base substitutions. The mean mutation frequency of six tumor samples was 2.2×10^{-6} . Individual mutation frequencies ranged from 0.6 to 4.8×10^{-6} , an average increase of greater than 200-fold compared with paired normal tissues. A dramatic increase in the frequency of random mutations has also been independently demonstrated in lung tumors with the RMC assay [32]. The diversity of nucleotide substitutions in tumor DNA is verified by DNA

sequencing, and indicates that each substitution is present in only one or a few cells, and that individual tumor cells contain more than 10,000 random mutations.

EMERGENCE OF RESISTANCE TO TARGETED THERAPIES

As a result of a mutator phenotype, tumors are likely to contain cells with mutant genes that render them resistant to any therapy directed against the tumor. It should be noted that, even with this high mutational load, there would be very few cells within a tumor with mutations rendering them resistant to multiple independent agents [33]. Thus, the presence of large numbers of random mutations in tumors provides a theoretical argument for the simultaneous or sequential administration of multiple therapies, which, based on empirical clinical evidence, has already become standard of care in most cancer types.

The emergence of resistance to imatinib in patients with chronic myelogenous leukemia (CML) provides clear evidence for the involvement of random mutations in drug resistance. Imatinib, commercially known as Gleevec, is an exceptionally effective single agent that specifically targets the BCR-ABL1 protein kinase and has become the primary treatment for CML [34]. Resistance, however, develops in more than 30% of patients and is mediated by point mutations in the ATP binding site [35]. This resistance can be overcome by administration of a second drug, e.g. Nilotinib, that targets a different site on the BCR-ABL1 protein kinase; however further resistance is mediated by the emergence of additional point mutations, which, when present in the same molecule, confer resistance to both drugs [36]. Evidence suggests that many of these resistance mutations were present in bone marrow before the onset of therapy [35]. Mathematical models of resistance in CML suggests that most of the imatinib resistance occurring within 2 years of therapy can be accounted for by preexisting resistance if one assumes a mutation rate of 10^{-8} /base per cell division [37, 38]. Additionally, prostate cancer metastases, analyzed after treatment with anti-androgens, reveal a variety of mutations in the androgen receptor (AR) gene which can alter promoter preferences, RNA and protein stability and ligand specificity [39]. Similar to the BCR-ABL1 resistance mutations in CML, these mutations in the androgen receptor gene are present at low frequency prior to selection with anti-androgens, again underlining the importance of a mutator phenotype to the emergence of resistance to targeted therapies.

TARGETING THE MUTATOR PHENOTYPE

The large numbers of mutations present in human tumors indicates that the malignant phenotype is irreversible. As a result, chemotherapy of cancer has been directed at identifying compounds that selectively kill cancer cells or drive them into senescence. Success has been limited in large part due to the multiplicity of proteins that can functionally substitute for those targeted by specific chemotherapies and by the subclonal and random mutations in a tumor that provides a reservoir for the emergence of resistance to any given therapy. As a common feature of most cancers, the mutator phenotype may be an exploitable target for therapy. Targeting the increased rate of mutations in cancer cells could conceivably be accomplished by two alternative approaches as illustrated in Fig. (1). First, it may be possible to reduce the incidence of mutation in precancerous diseases or during carcinogenesis to delay the onset or development of cancer. Second, increasing the rate of

mutation in cancer may incapacitate or kill malignant cells. The selection of the approach may be dependent on the rate and/or frequency of mutations in different tumors.

CANCER TREATMENT BY DELAY (PREVENTION)

For most solid human tumors, it takes 20 or more years from the time an individual is exposed to a carcinogen to the time that a cancer is detected clinically [40]. Clearly, preventing the initial mutagenic event, which may be as simple as changing behaviors to reduce exposure to carcinogens, is of primary importance in cancer prevention. However, if mutation accumulation results primarily from some endogenous condition, such as a chronic infection, a defect in metabolism, or a deficit in DNA repair, then effective strategies targeting the source of mutation would require new approaches to cancer therapy. If, as we assume, mutagenesis is rate-limiting for the phenotypic changes that are progressively evolving during tumor growth, it may be possible to reduce mutation accumulation, perhaps even prior to the clinical appearance of the tumor. Prevention is of particular importance in individuals with hereditary cancer syndromes or cancer predisposing conditions. Even after the initiation of a tumor or after clinical detection it may be feasible to delay the development of malignancy. A mere 2-fold reduction in the mutation rate may have a major impact on cancer mortality. Consider the following situations: 1) hepatitis B virus infects children in infancy, but results in hepatocellular carcinoma and death at a median age of 55 [41]; 2) prostatic hypertrophy at a median age of 50 is a biomarker for prostate cancer that results in death at age 85 [42]. In both of these cases, a 2-fold reduction in mutation rate would result in a marked reduction in cancer-related death in affected individuals. If, as we hypothesize, both carcinogenesis and development of drug resistance are dependent upon subclonal heterogeneity, reducing mutagenesis could prevent the further acquisition of competitively advantageous traits.

Chronic inflammation is associated with the early development of many cancers, and represents a potential target for reducing mutation induction in precancerous tissues. Chronic inflammation often precedes the appearance of cancer, notably in Barrett's esophagus, UC and Crohn's disease in colorectal cancer, and *Helicobacter pylori* infection in gastric cancer [43–45]. Aberrant regulation of immune pathways, often controlled by NF- κ B, its transcriptional targets, or upstream effectors, has been reported in these cancers, and the interplay between affected cells and immune cells promotes enhanced tumor growth [46, 47]. In addition to mitogenic effects, chronic inflammation may directly cause mutation or genetic instability. A recent report showed that chronic inflammation in the mouse colon led to an increase in precancerous lesions that was exacerbated by the depletion of the base excision repair (BER) glycosylase, alkyladenine DNA glycosylase (Aag) [48]. The spectrum of mutations observed in the *Aag*^{−/−} mice is consistent with that expected in response to reactive oxygen and nitrogen species generated by the immune cells, suggesting a link between inflammation and increased mutagenesis. Halogenated oxygen species produced by neutrophils and eosinophils also contribute to aberrant patterns of methylation, which can be viewed as functionally equivalent to mutation [49]. Some adducts can block the binding of DNA methyltransferase 1 from legitimate targets of methylation, while others, like 5-chlorocytosine, mimic methylated cytosine and encourage unintended methylation. Thus, treating and reducing chronic inflammation could delay the development of disease in

preneoplastic tissues, both by decoupling the mitogenic signaling loop between immune cells and transformed cells, and by reducing the accumulation of (epi)mutations.

LETHAL MUTAGENESIS AS AN APPROACH TO CHEMOTHERAPY OF ADVANCED CANCERS

An alternative approach is to increase the mutation rate in tumors. Inducing an error catastrophe in cancer cells, similar to that achieved with RNA viruses and retroviruses, suggests that enhanced mutagenesis may also be an option for therapy of specific tumors. This approach, termed lethal mutagenesis, has been used successfully in cultured cells to abolish infections with riboviruses [50] and human immunodeficiency virus (HIV) [51]. RNA-based viruses have an exceptionally high mutation rate (1×10^{-4}) bestowing great adaptability at the cost of viability; the vast majority of HIV progeny particles produced from an infected cell are unable to infect or reproduce, while the viable fraction is marked by genomic and phenotypic heterogeneity [52]. Populations of virus that mutate at high frequencies are maintained as quasispecies, composed of a spectrum of mutants that may include rapid and slow propagating variants, defective viruses, as well as drug-resistant and -sensitive viruses [53–55]. In a quasispecies, vigorously replicating viruses assure the survival of the population, while the poorly replicating viruses provide genetic flexibility to facilitate survival within, and rapid adaptation to, a changing selective environment. The concept of a quasispecies may also be applicable to tumors containing a population of cells that have accumulated large numbers of random, independent mutations.

The HIV population in an infected individual appears to exist near the brink of extinction. Initial studies with the mutagenic nucleoside analog, 5-OH-deoxycytidine, demonstrated that collapse of the viral population could be achieved in as few as 9 passages in serial transfer studies with HIV-infected human cells [51]. Unlike chain-terminating deoxy-nucleotides commonly used for HIV therapy, which have altered sugar moieties that inhibit replication, mutagenic deoxynucleosides have alterations at base positions that permit fraudulent base pairing during replication by the viral reverse transcriptase. In these experiments, viruses obtained in the penultimate passage exhibited a 2-fold increase in the frequency of random single-base substitutions that is in accord with the base pairing properties of 5-OH-deoxycytosine. These studies led to the development of the drug 5-aza-5,6,-dihydro-2'-deoxycytidine (KP-1212, Koronis Pharmaceuticals), which abolishes HIV infection at a concentration 100-fold less than that of 5-OH-dC during serial transfer experiments. HIV obtained from infected cells incubated with KP-1212, or from HIV carriers who had been administered the prodrug KP-1461 (metabolized in the liver to KP-1212) revealed predominantly A→G and G→A single-base substitutions, as would be predicted from the base-pairing properties of KP-1212 ([56], Mullins *et al. unpublished data*).

The human body is a hostile environment to tumor cells, which benefit from a suite of mutations that permit abnormal cell divisions and could account for resistance to killing by chemotherapy. Strong selective pressure, such as the presence of anti-cancer agents or a robust immune response, favors cancers with a higher mutation rate and greater phenotypic diversity. As in the case of HIV, we hypothesize that cancer cells operate within a limited tolerance for additional mutations, achieved by balancing flexibility with viability. Although

the likelihood of normal and cancer cells acquiring a dominant lethal mutation may be equal per cell division, the large number of acquired somatic alterations in cancer cells make the likelihood of acquiring a synthetically lethal mutation much higher in cancer cells. An increase in the mutation rate in tumor cells should therefore be more detrimental than a similar increase in the mutation rate in normal cells.

COMPOUNDS FOR LETHAL MUTAGENESIS

Since the initial discovery of 5-fluorouracil more than fifty years ago [57], a variety of nucleoside analogs have been used in the treatment of human cancers. Many of these have been selected based on termination of DNA chain elongation, or on inhibition of enzymes involved in deoxy-nucleoside metabolism such as thymidylate synthase and ribonucleotide reductase. The unscheduled termination of DNA replication by stalled replication forks leads to DNA breaks, cell cycle arrest, and potentially apoptosis [58]. Other agents that form DNA adducts may also interact with free deoxynucleosides and alter their base pairing properties, or perturb nucleotide pools. Among these are alkylating agents used in the treatment of acute leukemias and glioblastomas, and ionizing radiation that induces DNA breaks. The response elicited by each of these therapies may involve the induction of mutations; however, mutagenesis is usually considered to be an undesirable outcome, and has not been the focus of direct screening for effective chemotherapeutic agents.

Mutagenic nucleosides are an attractive class of lead molecule for lethal mutagenesis, provided they can be designed to efficiently introduce mutations that are not rapidly repaired. Effective nucleoside analogs as candidates for lethal mutagenesis should possess the following attributes: first, the analog should be stable and water soluble; second, nucleoside analogs should harbor small substituents on the bases so that they can be easily incorporated by DNA polymerases. These alterations should favor the formation of hydrogen bonds with non-complementary bases at high frequency. Third, the analog should be rapidly transported into human cells and phosphorylated by cellular nucleoside and nucleotide kinases. Fourth, the resulting deoxynucleoside triphosphate needs to be incorporated into DNA by the major replicating DNA polymerases, Pol- δ or Pol- ϵ , even in competition with the corresponding unaltered nucleoside triphosphate. Lastly, the analog should be resistant to detection or excision by either BER or MMR processes. Mutagenic nucleoside analogs that meet these criteria could encompass both natural purines and pyrimidines as well as those with novel chemical structures.

POTENTIAL SHORTCOMINGS OF LETHAL MUTAGENESIS

Clearly, there are important concerns associated with increasing the frequency of random mutations in human cancers. Mutagenic nucleotides may also be incorporated into DNA in non-malignant cells, form mispairs at the time of DNA replication, and cause mutations. There is no Reason, *a priori*, to assume these agents will be preferentially incorporated into tumor cells, even though incorporation of analogs may be more frequent due to pre-existing mutations that widen the nucleotide binding pocket in DNA polymerase, or mutations that deregulate DNA repair processes. There are three factors that might mitigate the consequences of increasing mutations in normal cells. First, a small increase in the

frequency of random mutations in cancer cells may be sufficient to induce lethal mutagenesis since these cells have already accumulated many mutations, and are thus closer to an error threshold. In contrast, mutations are much less frequent in normal cells and a greater degree of mutagenesis may be required to achieve a threshold of critical mutations. Second, it takes many years from the time of exposure of an individual to a mutagen to the clinical appearance of a tumor, so the induction of new cancers in treated individuals may not be a primary concern. Third, it is likely that normal cells will be more sensitive to the presence of DNA adducts and nucleotide pool imbalances than cancer cells, and would either repair or arrest with greater efficiency than cancer cells, minimizing any mutagenic consequences of treatment.

Some recent efforts in the design of chemotherapeutic drugs have targeted DNA damaging agents to cancer cells that are sensitized to that type of damage, based either on the cellular phenotype, or on the effects of an inhibitor. For example, current clinical trials for glioblastoma are centered on poly (ADP ribose) polymerase (PARP-1) inhibitor and combined Temozolomide (TMZ)/radiation. By inhibiting PARP-1, cells are less able to repair mismatch repair-based DNA damage introduced by TMZ, and the radiation helps to convert damaged DNA into double-stranded breaks. Another example is the selective killing of aneuploid cancer cell lines by inhibiting the mitotic spindle checkpoint with siRNAs against Mps1 and BubR1 in combination with low dose Paclitaxel (taxol) treatment, which inhibits microtubule dynamics [59]. Preexisting aneuploidy appears to be necessary to use this strategy effectively, as similarly-treated immortalized diploid fibroblasts that maintained ploidy had no synergistic or additive susceptibility to taxol-induced cell death when Mps1 expression was knocked down. Thus, normal cells could conceivably tolerate a mutagenic strategy by virtue of intact DNA repair and recognition capacities.

EXPLOITING THE MUTATOR PHENOTYPE FOR DIAGNOSIS AND THERAPY

While there are no clear molecular targets to reduce the rate of mutation accumulation, the link between inflammation and mutation suggests that anti-inflammatory therapy may be useful in delaying the onset or development of cancer from high-risk, precancerous tissues. Our work on mapping mutated microsatellite sequences in the colon using standard endoscopic biopsies has revealed a powerful predictive tool for pinpointing likely areas of malignancy in patients with ulcerative colitis, and could provide a meaningful marker for tracking the effectiveness of such therapy [20]. There is a need for diagnostics to identify the underlying molecular defect(s) behind the mutator phenotype in a given cancer. A clear deficiency in a DNA repair pathway can point the way to an effective therapy, either to directly kill the cells by inducing widespread DNA damage or to induce lethal mutagenesis [60]. A variety of studies have determined that a highly microsatellite unstable (MSI-H) phenotype is associated with a more poorly differentiated cancer, but also with better overall prognosis than a microsatellite stable (MSS) phenotype [61–63]. One hypothesis for this seeming paradox is that MSI-H cells, having acquired a large number of alterations, are more susceptible to the introduction of synthetic lethal mutations, and the only recourse against this is to lose the MSI-H phenotype. Additionally, the greater genomic instability in MSI-H cells leads to a greater frequency of immunogenic epitopes in the affected cells.

Thus, enhancing mutagenesis, in addition to increasing the odds of introducing synthetic lethal mutations, may also enhance the host immune response.

Due to the potential undesirable consequences associated with the use of lethal mutagenesis, particularly risk of secondary tumors, this new approach to cancer therapy would be applicable to only specific circumstances. We envision this strategy used in combination with other agents 1) for cancers that have not responded to chemotherapy, or that have become resistant to all other effective agents, and 2) in elderly individuals in which the induction of new tumors is less likely to be consequential. Measurements of a number of pre-existing mutations in individual tumors also may be an important determinant to assess the utility of mutagenic nucleotides as therapy. Despite the potential risks, the use of mutagenic therapy may be attractive considering the limited number of options available to many patients.

Acknowledgments

We thank Drs. Ray Monnat, Alan Herr, James Shen and Scott Kennedy for critical reading of the manuscript and members of our laboratory for helpful discussions.

GRANT SUPPORT

NIH (CA102029 and CA78885) and DOD (W81XWH) grants to L.A. Loeb; National Cancer Institute/Health Research Board of Ireland Joint Research Fellowship (JRFC-05-01) to E.J. Fox.

ABBREVIATIONS

AAG	Alkyladenine DNA glycosylase
AML	Acute myeloid leukemia
BER	Base excision repair
CML	Chronic myelogenous leukemia
HIV	Human immunodeficiency virus
HNPCC	Hereditary non-polyposis colorectal cancer
MMR	Mismatch repair
PARP	Poly (ADP ribose) polymerase
TMZ	Temozolomide
UC	Ulcerative colitis

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Figure 1. Targeting the mutator phenotype in cancer

Over time, untreated cancer accumulates mutations (red cells), contributing to heterogeneity and promoting carcinogenesis. A cancer initiating event occurs at 0 years. Diversity of the cancer cell population is represented by different shapes of nuclei in the cells, and tumor burden is represented by the size of the cell clusters. Reducing the rate of mutation, or **Treatment by Delay**, could prolong the time to tumorigenesis by a significant degree in some cancers (green cells). Accelerating the accumulation of mutations beyond the rate favored by selection, or **Lethal Mutagenesis**, could rapidly drive cells over an error threshold, increasing the chance that most cells will acquire a lethal mutation (yellow cells).

Table 1

Summary of Data from Select Cancer Genome Sequencing Studies

Tumor	(n)	Genes Sequenced	Total Mutations(Tumor)	Non-Silent Mutations per Tumor	References
Breast	11	18,191	1,243	84	[12, 22]
Colorectal	11		942	76	
Diverse	210	518	798	NA	[64]
Pancreatic	24	20,661	1,163	48	[10]
Glioblastoma	210	20,661	748	47	[65]
Glioblastoma	91	601	453	NA	[66]
Lung	188	623	1,013	NA	[67]
Acute Myeloid Leukemia	1	Whole Genome	500–1,000	10	[24]
Acute Myeloid Leukemia	1	Whole Genome	750	12	[25]
Lung	1	Whole Genome	>33,345	188	[27]
Malignant Melanoma	1	Whole Genome	>22,910	100	[26]