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Human Cancers Express a Mutator Phenotype: Hypothesis, Origin, and Consequences

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Abstract

The mutator phenotype hypothesis was postulated more than 40 years ago. It was based on the multiple enzymatic steps required to precisely replicate the 6 billion bases in the human genome each time a normal cell divides. A reduction in this accuracy during tumor progression could be responsible for the striking heterogeneity of malignant cells within a tumor and for the rapidity by which cancers become resistant to therapy.

Cancer is a disease of mutations resulting from endogenous and environmental damage to genomic DNA. The damage is distributed randomly throughout the genome. Unrepaired damaged sites in DNA can direct the misincorporation of nucleotides into daughter DNA strands during each cell division. Some of the generated mutations impart selective growth advantage by overcoming geographical constraints imposed by neighboring tissues, diminished vascular supply, and altered nutrition. By the time a tumor is clinically detected, there are tens to hundreds of thousands of different mutations; clonal lineages within the tumor are geographically demarcated and different genes are mutated in different individuals with the same types of tumors (1). This extensive landscape of subclonal mutations could account for the phenotypic heterogeneity of tumors as well as resistance to therapy (2).

The concept of the progressive accumulation of mutations in human cancers was initially presented as “The Mutator Phenotype Hypothesis” (3). It was formulated 40 years ago based on: (i) the precision of multiple enzymatic steps required to duplicate the genome in normal cells; (ii) the free energy difference between complementary and noncomplementary base pairing is only 1 to 3 kcal, and thus insufficient to account for the mutation rate of one error in 10^9 nucleotides per each cell division. There had to be other factors that enhance the accuracy, and there was evidence then for the involvement of DNA polymerases in facilitating accurate base selection and base pairing; mutations in DNA polymerases could reduce the fidelity of DNA replication (4); (iii) human inherited diseases with a high proclivity to developing malignancies were already known to be associated with deficits in DNA repair (5); (iv) chromosomal mutations were also known to accumulate during tumor

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progression; (v) many cancers rapidly acquire resistance to chemotherapy agents. The overall concept was that mutations occurred randomly throughout the genome, and among these would be mutations in genes that guarantee the fidelity of DNA replication.

Initially, the focus was on mutations that would render replicative DNA polymerases error-prone. With each round of DNA replication, there would be an exponential increase in the number of mutations. The suggestion that each malignant cell within a tumor could contain tens of thousands of subclonal mutations and that a tumor may have as many as 1 billion different mutations was taken with disbelief when presented at meetings (6). With the increasing realization that normal cellular processes as well as environmental agents extensively damage the cellular genome (7), the focus was expanded to include mutations in DNA repair genes.

Until recently, the concept of a mutator phenotype was not widely considered as a major contributor to tumor initiation or progression. Instead, the most prevalent concept was sequential selection of mutations in different genes that impart proliferative advantage under restrictive conditions (8). This was particularly attractive because it inferred a few druggable therapeutic targets. Furthermore, it was argued that malignant cells need not have increased mutations rates compared with normal cells (8), as only one or a few mutant genes are sufficient to induce tumor proliferation in *in vitro* model systems. It was proposed that a greater number of stem cell divisions would be adequate to account for mutation accumulations. The correlation between stem cell division and risk of carcinogenesis has become highly controversial (9).

Ironically, the strongest evidence in support of the mutator phenotype in human tumors is data presented by The Cancer Genome Atlas (TCGA), which was designed to identify mutations in a few genes that might be exploited for chemotherapy. As more and more tumors were analyzed, the number of mutations in each tumor was established. Currently, the number of observed mutations per tumor ranges from 500 in acute myelogenous leukemia to 100,000 in melanomas and glioblastomas (10). Most of these mutations were detected by routine next-generation DNA sequencing, in which mutations are scored only if present at >1%–5% frequency, and thus likely to be clonal mutations, that is, those that are present in the majority of cells within a tumor. To investigate heterogeneity within tumors, it is necessary to identify subclonal mutations, those that are present in only a small fraction of cells. We recently established a methodology that offers unprecedented accuracy, referred to as Duplex Sequencing (11). By sequencing both strands of the same molecule and defining mutations as complementary substitutions present at the same position in both strands, one can achieve accuracy of 10^{-8}, which is at least 1,000-fold more accurate than routine next-generation sequencing. We find in some tumors that there are more subclonal than clonal mutations (unpublished results). This extensive landscape of subclonal mutations could account for the phenotypic heterogeneity of tumors.

Sources of Mutations

The origin of multiple mutations in tumors has not been fully established. A major clue comes from the spectrum of mutations identified in many tumors. A compilation of various

databases of mutant tumor DNA sequences indicates that single-nucleotide substitutions predominate. Single-nucleotide substitutions might act as passenger mutations that do not alter cellular phenotypes and thus could be well tolerated. Deletions, insertions, and rearrangements are more likely to be either detrimental or lethal. The most frequent clonal and subclonal single-nucleotide substitutions in human tumors as well as in normal tissues are transition mutations—G:C to A:T or A:T to G:C substitutions (G:C to A:T are >60% of mutations). On the basis of biochemical studies, the most likely sources are misincorporation by DNA polymerases or spontaneous deamination of cytosine to uracil. Other potential sources of single-nucleotide mutations include alterations in deoxynucleoside triphosphate pools, incorporation of ribonucleotides into DNA, and mutations in base excision and mismatch repair genes.

Mutations in DNA Polymerases

DNA polymerases are the enzymes responsible for the accurate replication of 6 billion nucleotides each time a cell divides, as well as for the resynthesis of DNA after damage bases are removed. It was initially postulated that mutations in DNA polymerases would diminish the accuracy of DNA synthesis, resulting in single-nucleotide substitutions scattered throughout the genome. The genes encoding replicative DNA polymerases are among the most conserved DNA sequences in evolution. The findings that alterations in either the polymerase or exonuclease (proofreading) domains can affect the fidelity of DNA synthesis *in vitro*, and that mice harboring mutator DNA polymerases that lack proofreading activity exhibit an increased incidence of cancers, suggested that mutations in genes encoding polymerases could be a source for multiple mutations in human tumors. However, until recently, there was only a single report of a mutation in a replicative DNA polymerase, and it was in an extensively passaged cell line (12). Presumably, heterozygous mutations would diminish the rate of DNA synthesis, and homozygous mutations would be lethal. This was in accord with the lack of finding these mutations in the initial studies.

Within the last 3 years, reevaluation of the TCGA database indicates that 16% of colorectal tumors are hypermutators and that one-fourth of these tumors have mutations in the proofreading domain of *POLD1* or *POLE* (which encode the catalytic subunits of replicative DNA polymerases, Pol δ and Pol ε, respectively; ref. 13). Germline mutations found at these sites predispose individuals to colorectal cancer. Somatic mutations have also been found in endometrial cancers and occasionally in cancers of the breast, stomach, pancreas, and brain (14). By far, the highest mutation frequencies observed occur in pediatric brain tumors with biallelic mutations in mismatch repair genes and acquired mutations in the proofreading domain of *POLD1* or *POLE* (15). The mutation frequency approaches 10^{-3}; any further increase might be incompatible with cell viability. In addition to replicative DNA polymerases, there are specialized cellular polymerases that are recruited to stalled replication forks to facilitate bypass of blocking DNA lesions; these bypass polymerases are error-prone and could also contribute to the generation of mutations in tumors.

Extension of the Hypothesis to DNA Repair

The concept of the mutator phenotype was initially based on mutations in DNA polymerases and later extended to mutations in DNA repair genes. Xeroderma pigmentosum germline mutations in nucleotide excision repair are causally associated with a 1,000-fold increase in the incidence of skin cancers upon exposure to UV irradiation (5). Mutations in genes that encode mismatch repair result in a 100-fold increase in alterations and lengths of repetitive sequence and in colon cancer (16). Studies in yeast identified hundreds of genes that are required to maintain genetic stability (17), and if mutated, are candidates for the generation of mutations throughout the genome. Thus, there is ample evidence to extend the mutator phenotype hypothesis.

Controversies

The mutator phenotype hypothesis has been controversial for many years. There is the extreme technical challenge of accurately quantifying very rare mutations. Also, it is argued that the number of stem cell divisions is adequate to generate the large number of mutations found in human tumors without invoking an increase in mutation rates (9). The ultimate proof will require measuring mutation rates in single cells or single molecules at multiple sites in the genome in the absence of selection. Duplex Sequencing offers the sensitivity and accuracy to approach this problem.

Until recently, the most accepted hypothesis was that tumors evolve by sequential rounds of selection for cells harboring specific mutations. During tumor progression, there would be a sequence of selective clonal sweeps for mutations that allow growth under restrictive conditions. There is indeed evidence for a temporal order of mutations in key cancer-associated genes after the clinical appearance of colorectal and pancreatic cancers. However, it has not been feasible to investigate sequential mutations before tumors are detected. Moreover, the concept of sequential expression of mutant genes does not account for the tens of thousands of mutations observed in most human tumors, nor that geographically distinct regions of malignant cells within the same tumors have different mutations. Nonetheless, cells with common mutant genes within a tumor offer an attractive target for therapy.

Targeting a Mutator Phenotype

Two divergent protocols have been advanced for reducing the inevitability of mutation accumulations in human cancers. As mutation accumulation is likely rate limiting for tumor progression, a reduction in the overall fitness of the tumor cell population could lengthen the interval between the initiation of cancer and its clinical sequelae. For most human cancers, there is a 5- to 40-year interval between the exposure of an individual to a carcinogenic agent and the detection of malignancy. For example, hepatitis B infection in the developing world usually occurs before adolescence, yet the median age of presentation of hepatocellular carcinoma is 45 years. If one could double the number of years for tumor cells to accumulate mutations required for proliferation, invasiveness, or metastasis, one

could delay the clinical manifestations of the cancer. Thus, a 2-fold reduction in mutation accumulation could have dramatic effects.

The evolutionary success of many RNA viruses has been attributed to the persistent generation of diversity within the viral population. An increase in mutation rates by incorporation of nucleoside analogues that enhance mutagenesis extinguishes RNA viruses. It has been proposed that some human cancers can also be selectively ablated by the incorporation of mutagenic nucleoside analogues (18). The preexisting mutational load of many human cancers may be close to the threshold for viability. In addition, drugs used to treat cancer patients damage DNA and are mutagenic, further elevating the mutation load of tumors. Recent studies in yeast demonstrate that even the lethal effects of the mutator phenotype can be negated by the evolution of antimutator clones (19). Thus, treatment of patients with nucleoside analogues may only be applicable to select patients; it will have to be carefully monitored.

In conclusion, the mutator phenotype was formulated more than 40 years ago. Only now have advances in basic research permitted us to determine its role in tumor evolution.

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References

1. De Grassi A, Segala C, Iannelli F, Volorio S, Bertario L, Radice P, et al. Ultradeep sequencing of a human ultraconserved region reveals somatic and constitutional genomic instability. PLoS Biol. 2010; 8:e1000275. [PubMed: 20052272]
2. Beckman RA, Schemmann GS, Yeang C-H. Impact of genetic dynamics and single-cell heterogeneity on development of nonstandard personalized medicine strategies for cancer. Proc Natl Acad Sci U S A. 2012; 109:14586–91. [PubMed: 22891318]
3. Loeb LA, Springgate CF, Battula N. Errors in DNA replication as a basis of malignant changes. Cancer Res. 1974; 34:2311–21. [PubMed: 4136142]
4. Kunkel TA, Loeb LA. Fidelity of mammalian DNA polymerases. Science. 1981; 213:765–7. [PubMed: 6454965]
5. Cleaver JE. Defective repair replication of DNA in xeroderma pigmentosum. Nature. 1968; 218:652–6. [PubMed: 5655953]
6. Klein CA. Random mutations, selected mutations: a PIN opens the door to new genetic landscapes. Proc Natl Acad Sci U S A. 2006; 103:18033–4. [PubMed: 17116890]
7. Lindahl T, Wood RD. Quality control by DNA repair. Science. 1999; 286:1897–905. [PubMed: 10583946]
8. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990; 61:759–67. [PubMed: 2188735]
9. Tomasetti C, Vogelstein B. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science. 2015; 347:78–81. [PubMed: 25554788]

10. Alexandrov LBB, Nik-Zainal S, Wedge DCC, Campbell PJJ, Stratton MRR. Deciphering signatures of mutational processes operative in human cancer. Cell Rep. 2013; 3:246–59. [PubMed: 23318258]
11. Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, Loeb LA. Detection of ultra-rare mutations by next-generation sequencing. Proc Natl Acad Sci U S A. 2012; 109:14508–13. [PubMed: 22853953]
12. Flohr T, Dai JC, Büttner J, Popanda O, Hagmüller E, Thielmann HW. Detection of mutations in the DNA polymerase delta gene of human sporadic colorectal cancers and colon cancer cell lines. Int J Cancer. 1999; 80:919–29. [PubMed: 10074927]
13. The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012; 487:330–7. [PubMed: 22810696]
14. Rayner E, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I, et al. A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer. 2016; 16:71–81. [PubMed: 26822575]
15. Shlien A, Campbell BB, deBorja R, Alexandrov LB, Merico D, Wedge D, et al. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. Nat Genet. 2015; 47:257–62. [PubMed: 25642631]
16. Kim T-M, Laird PW, Park PJ. The landscape of microsatellite instability in colorectal and endometrial cancer genomes. Cell. 2013; 155:858–68. [PubMed: 24209623]
17. Kolodner RD. Mismatch repair: mechanisms and relationship to cancer susceptibility. Trends Biochem Sci. 1995; 20:397–401. [PubMed: 8533151]
18. Fox EJ, Loeb LA. Lethal mutagenesis: targeting the mutator phenotype in cancer. Semin Cancer Biol. 2010; 20:353–9. [PubMed: 20934515]
19. Herr AJ, Kennedy SR, Knowels GM, Schultz EM, Preston BD. DNA replication error-induced extinction of diploid yeast. Genetics. 2014; 196:677–91. [PubMed: 24388879]