DNA Repair 56 (2017) 7–15

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Review

Evolutionary dynamics and significance of multiple subclonal mutations in cancer

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ARTICLE INFO

Keywords:
Cancer
Mutator hypothesis
DNA replication
DNA repair
Carcinogenesis
Evolutionary dynamics
Duplex sequencing
Mathematical models

ABSTRACT

For the last 40 years the authors have collaborated on trying to understand the complexities of human cancer by formulating testable mathematical models that are based on mutation accumulation in human malignancies. We summarize the concepts encompassed by multiple mutations in human cancers in the context of source, accumulation during carcinogenesis and tumor progression, and therapeutic consequences. We conclude that the efficacious treatment of human cancer by targeted therapy will involve individualized, uniquely directed specific agents singly and in simultaneous combinations, and take into account the importance of targeting resistant subclonal mutations, particularly those subclones with alterations in DNA repair genes, DNA polymerase, and other genes required to maintain genetic stability.

1. Introduction

Until some twenty years ago it was commonly accepted that every cell in our body contained similar, if not identical, nuclear genomes. Obviously, there were exceptions– repetitive elements that expanded and contracted, line elements that duplicated, and telomeres that shrunk and elongated. But these were the exceptions. In contrast, the interrogation of multiple genomes from the same individual by massively parallel next generation DNA sequencing (NGS) provided evidence of extensive multigenic mosaicism in cancers, and even in non-malignant tissues. The low accuracy of routine NGS precluded the detection of rare subclonal mutations and prevented us from realizing the unanticipated plasticity of our genomes.

The recent award of the Nobel Prize in Chemistry for DNA repair brought into focus the extensiveness of DNA damage that occurs in human cells. Advances in chemistry have greatly extended our knowledge of the enzymology of DNA repair and the structure of intermediates in DNA damage repair; advances in DNA sequencing are making it possible to approach exciting biological questions surrounding DNA damage and mutagenesis. Tomas Lindahl calculated that each cell in our body undergoes some 50,000 DNA damage events per day [1]. There are multiple scenarios that could occur when the DNA replicating and/or repair apparatus encounters unrepaired DNA damage. Small lesions are frequently bypassed by DNA polymerases [2]. Larger DNA adducts are more likely to stall DNA replication [3], induce the SOS-response in bacteria, and increase the expression of the Y- family DNA polymerases in eukaryotic cells [4–6]. These specialized DNA polymerases have active sites that can encompass bulky lesions [7], allowing DNA synthesis to proceed.

The accuracy of DNA repair is governed by the ability of repair complexes to recognize distortions in DNA resulting from the presence of altered nucleoside bases and sugar residues. The pioneering work in the laboratory of Phil Hanawalt, along with that of others, established pathways for nucleotide excision repair, global excision repair [8] and transcription coupled repair [9], and consequences of deficiencies in these processes. The fact that biallelic mutations that inactivate many DNA repair enzymes are lethal substantiates the importance of DNA repair mechanisms [10]. Minor changes in the structure of these proteins can result in decreased fidelity of DNA repair processes.

The accuracy of DNA replication also depends on both initial conformational recognition of correct base-pairs by the polymerase active site and subsequent proofreading steps [11–13]. The work of Sam Wilson established alterations in the structure of DNA polymerase β at the template-binding site as it encounters complementary or non-complementary nucleotides [14].

Until recently, DNA repair pathways and polymerases were not

Abbreviations: APC, adenomatous polyposis coli; DNA, deoxyribonucleic acid; FQM, focused quantitative modeling; HER2, human epidermal growth factor receptor 2; HIV, human immunodeficiency virus; NGS, next generation sequencing; Pol, polymerase; RF, reduced fitness; TCGA, The Cancer Genome Atlas

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http://dx.doi.org/10.1016/j.dnarep.2017.06.002

Available online 09 June 2017

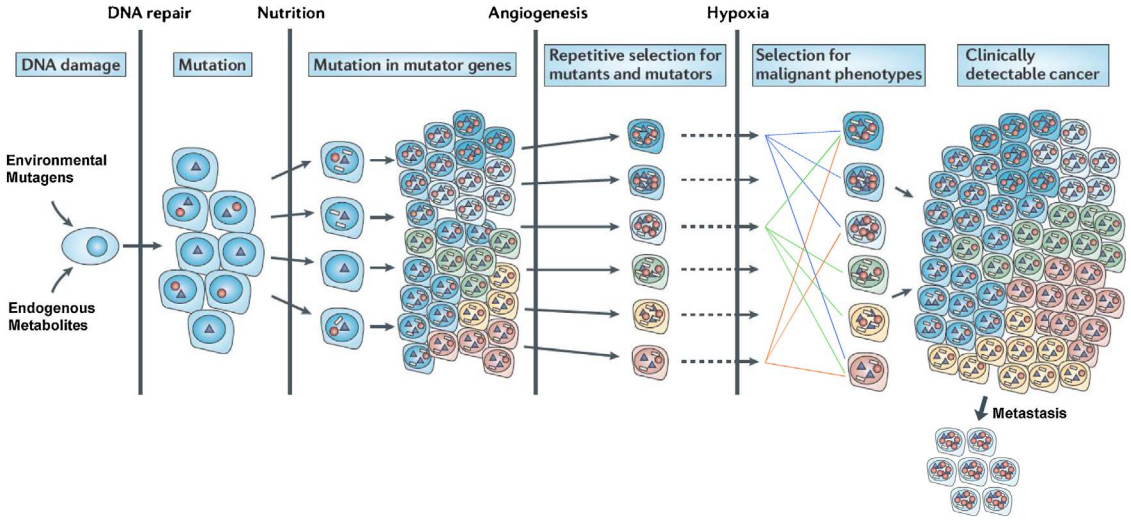

Fig. 1. Mutational cascade during carcinogenesis as envisioned by the mutator hypothesis. During tumor progression there is a progressive increase in mutations resulting from unrepaired DNA damage. Most have no effect on cellular phenotypes (neutral), others enhance proliferation (drivers), and others cause increased mutagenesis (mutators). As the tumor encounters environmental restrictions such as reduced nutrition, inadequate angiogenesis, hypoxia, etc. specific mutations are selected. Circles represent mutations in genes that enhance mutagenesis, triangles indicate mutations selected that enhance proliferation under adverse conditions, and white rectangles represent passenger mutations of unknown functions. Note that many of the tumor cells contain multiple drivers and mutators. Also to be noted is that many topographically distinct mutations are maintained during tumor proliferation. Adapted from [22].

considered as primary targets for cancer therapy. There were no reports of mutations in DNA polymerase genes in the extensive databases compiled by analyzing DNA from human tumors [15]. Reanalysis of the same database unexpectedly provided extensive documentation that the major replicating DNA polymerases, Pol-δ and −ε, were mutated in several human cancers [16]. Moreover, human colon cancers that have mutations in the Pol-δ or Pol-ε exonuclease domain have exceptionally high mutation frequencies throughout their genomes [17,18]. Patients with brain tumors that carry inherited biallelic mismatch repair mutations and develop somatic mutations in replicative DNA polymerases have the highest mutation frequencies reported, (> 250/Mb) [19]. Similarly, only a limited number of mutations were reported in the multiple DNA repair pathways. However, recent reports indicate that 10% of metastatic prostate cancers contain inherited mutations in DNA repair genes [20]. From these studies we conclude that DNA repair and DNA replication proteins are attractive targets for design of inhibitors of proliferation in human cancers.

This article presents our perspective on the association of spontaneous mutations with the initiation and progression of human cancers. It stems from collaborations that have been ongoing for more than 40 years on the pathways by which damaged DNA results in mutations in normal and malignant cells, including constructing mathematical models that probe the mechanism and the consequences of mutation for carcinogenesis, tumor evolution, and therapy. The mutator hypothesis [21] states that tumors are genetically unstable compared to normal tissue, and that this plays a critical role in carcinogenesis. The hypothesis of a mutator phenotype in human cancer is increasingly supported by the power of DNA sequencing to unveil the thousands and perhaps millions of changes in the nucleotide sequence of DNA present in the genomes of many cancer cells [22].

In this commentary, we will focus on mutational diversity. We note that DNA mutations (single-base substitutions) are not the only clinically relevant source of phenotypic variation in human cancers. Chromosomal rearrangements, gene amplification, and stable epigenetic changes can also cause long-term phenotypic variation. Furthermore, short-term plasticity in gene expression can cause transient phenotypic variation within stable epigenetic or genetic states [23]. These latter phenomena are clearly important and can rapidly cause resistance to targeted therapy in a majority of cells within a tumor. The studies carried out in cultured cells and small animals are frequently of short duration and involve fewer cells compared to clinical cancers, and thus may preferentially score for the rapid onset of resistance that is often reversible. Resistance to therapy in humans resulting from DNA alteration is permanent, is observed late in the growth of tumors, and is likely to represent the emergence of pre-existing subclonal mutations [24].

2. Experimental support for the mutator phenotype hypothesis

2.1. Mechanistic studies of DNA replication, damage, and repair

Originally, the mutator hypothesis was framed around errors made by DNA polymerases during DNA replication [21]. However, with growing knowledge of DNA replication and repair, it became apparent that there are hundreds of genes involved in these processes, alterations of which could also result in enhanced mutagenesis [25]. We envisioned a cascade of mutation accumulation in cancer cells manifested by increasing heterogeneity with random mutations accumulating in DNA replication and repair proteins. It was postulated that amongst the earliest molecular events that initiated transformation of normal cells into premalignant cells was damage to critical genes required for maintaining genetic stability. The initial focus was on replicative DNA polymerases (Pol −α, −δ, and −ε) [13]; these enzymes are responsible for the accurate copying of some 6 billion nucleotides during each division cycle. Single amino acid substitutions in their catalytic sites result in increased errors in nucleotide incorporation or decreased proofreading. Mutations that reduce the accuracy of nucleotide selection or of exonucleolytic hydrolysis of mis-incorporated nucleotides without diminishing rates of polymerization could result in increased single-base substitutions throughout the genome. Some of these polymerase-induced mutations could occur in additional genes required to maintain genetic stability. A cascade of mutations would ensue, resulting in progressive accumulation of mutations in human tumors (Fig. 1).

In humans, the earliest mutations characterizing the transformation of normal cells into malignant cells occur prior to diagnosis and can be extrapolated from DNA obtained from clinical samples. Even with tumors in animals we lack adequate technologies to detect and analyze the earliest changes. The 50,000 lesions produced per cell per day as a result of spontaneous and/or endogenous chemical reactions [1] are not localized to specific genes but instead are distributed stochastically.