Seminars in Cancer Biology 20 (2010) 312–319

Contents lists available at ScienceDirect

Seminars in Cancer Biology

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

Review

Aberrant expression of alternative DNA polymerases: A source of mutator phenotype as well as replicative stress in cancer

Jean-Sébastien Hoffmann*, Christophe Cazaux

CNRS, IPBS (Institute of Pharmacology and Structural Biology), 205, route de Narbonne, University of Toulouse, UPS, 31077 Toulouse, France

ARTICLE INFO

Keywords:
Mutator phenotype
Replication stress
Alternative DNA polymerases
Genetic instability

ABSTRACT

The cell life span depends on a subtle equilibrium between the accurate duplication of the genomic DNA and less stringent DNA transactions which allow cells to tolerate mutations associated with DNA damage. The physiological role of the alternative, specialized or TLS (translesion synthesis) DNA polymerases could be to favor the necessary "flexibility" of the replication machinery, by allowing DNA replication to occur even in the presence of blocking DNA damage. As these alternative DNA polymerases are inaccurate when replicating undamaged DNA, the regulation of their expression needs to be carefully controlled. Evidence in the literature supports that dysregulation of these error-prone enzymes contributes to the acquisition of a mutator phenotype that, along with defective cell cycle control or other genome stability pathways, could be a motor for accelerated tumor progression.

1. Genetic instability in cancer has a wide range of expression modes

Cells from solid cancers differ from normal cells in many important characteristics, including loss of differentiation, increased ability to invade, and decreased sensitivity to chemotherapeutic agents. In most cases, these differences do not arise simply from uncontrolled cellular growth, but rather from a genetic flexibility providing a continuing pool of mutants upon which selection could act to promote a tumor. Genetic instability in solid tumors is manifested in many ways: by chromosome aberrations (chromosomal instability or CIN), affecting chromosomal structure (producing translocations, sequence gains, or losses) or karyotypic integrity (resulting in aneuploidy), changes in the length of microsatellite sequences (microsatellite instability or MIN), as well as by clonal mutations, including mutations in oncogenes and tumor suppressor genes. The genomes of human cancer cells also display greatly elevated frequencies of random nucleotide point mutations, of which the minority is in protein-coding genes (point mutation instability or PIN) [1–5]. This strongly supports the concept of a mutator phenotype in human cancers formulated initially by Loeb [6–8], and which is still the subject of ongoing debate. So far, 113,287 different mutations associated with cancer have been reported (www.sanger.ac.uk/genetics/CGP/cosmic/). This hypothesis suggests that early events in tumorigenesis are mutations that inactivate a gene that normally functions to maintain genome stability, resulting in an elevated mutation rate. This elevated mutation rate in turn results in additional mutations in other genes associated with multistage carcinogenesis, which then confer selective advantages that allow mutated cells to expand and achieve clonal dominance. Such untargeted mutations (dispersed randomly) could contribute to the morphologic and functional heterogeneity of cancers and include mutations that confer resistance to therapy. Ovarian carcinoma with mutations in the tumor suppressor *BRCA2* acquire resistance to cisplatin and PARP inhibitor mediated by secondary intragenic mutations in *BRCA2* that restore the wild type *BRCA2* reading frame [9]. The emergence of resistance to imatinib (Gleevec) in patients with chronic myelogenous leukemia (CML) provides also clear evidence for the involvement of random mutations in drug resistance. Imatinib specifically targets the BCR-ABL protein kinase, and it has become the primary treatment for CML. Unfortunately, resistance develops in 30–90% of patients and is mediated by point mutations in the ATP binding site [10].

All these different manifestations of genetic instability are not mutually exclusive and may be mechanistically linked. The concept that cancer must exhibit an increased genome-wide mutation rate early in their evolution was initially proposed based on mutations in replicative DNA polymerases that render them error-prone, and mutations in DNA repair genes that decrease the ability of cells to remove potentially mutagenic DNA damages, increasing the probability for acquisition of oncogenic mutations [7]. Consistent with this hypothesis, point mutations in the proofreading domain of Polδ or Polε cause a mutator- and cancer phenotype in mice [11,12], strongly suggesting that unrepaired DNA polymerase errors contribute to carcinogenesis. The mutator phenotype hypothesis has been then extended to include the multiple 3R (replication, repair, recombination) genes [13] that function to maintain genetic

* Corresponding author. Tel.: +33 5 61 17 59 75; fax: +33 5 61 17 59 94.
E-mail address: jseb@ipbs.fr (J.-S. Hoffmann).

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doi:10.1016/j.semcancer.2010.10.001

stability. The genes encoding components of the multiple cell cycle checkpoints represent another category of genes that play a role in genetic stability and cellular evolution [14]. The efficiency and fidelity with which these various DNA transactions and checkpoint operate can be keys to the origins of cancer. The importance of these highly conserved pathways for limiting cancer risk is clearly illustrated by the greatly elevated cancer risk of patients bearing a germ line mutation in these genes. Hereditary forms of colon, breast, ovary and skin cancers are caused by mutations in mismatch repair (e.g., hMLH1), DNA break repair (e.g., BRCA1), nucleotide excision repair pathways (e.g., XP proteins), or in proteins affecting the capacity to replicate through UV DNA damage (e.g., Polη), respectively. In somatic cancers, such early mutations become probably "diluted" in the alterations that follow, making the relationship less obvious. However, it is very likely that genetic instability accelerates and even possibly initiates the proliferation of cancer cells by favoring the emergence of variant cells. Indeed, a controlled alteration of genes involved in genome maintenance promotes or favors carcinogenesis. By this mutator driving force, the genotypes of most cells within a tumor would not be identical, but would share at least one mutation in any number of the genes that ensure DNA fidelity. With waves of clonal selection and expansion, the tumor would evolve as a heterogeneous collection of cancer cells, all sharing the common feature of genetic instability and all having different, but frequently overlapping, patterns of oncogenic mutations.

2. Subtle equilibrium between accurate DNA synthesis and less stringent DNA damage tolerance

Genomic replication in normal cells is regulated by an 'origin licensing' mechanism that ensures that it occurs just once per cycle. Once cells enter the S-phase, the stability of DNA replication forks must be preserved to avoid susceptibility to DNA lesions or non-B DNA conformation. The requirement of faithful genome duplication in dividing cells makes DNA replication an important factor in limiting cancer risk. Analysis of origin firing proteins can facilitate accurate detection of colorectal cancer in stool [15] and many anticancer drugs target various aspects of DNA transactions during its replication.

The maintenance of genome integrity and the necessary adaptation to genotoxic stresses are two key elements for ensuring both cell survival and evolution of multicellular organisms. In both Procaryotes and Eukaryotes, the accurate replication of *undamaged* genomic DNA requires the action of "replicative" DNA polymerases, also named "high-fidelity" or "error-free" polymerases, the main actors at the replication forks. Possessing proofreading ability, these enzymes allow the nearly perfect duplication of an undamaged genome. In human cells, this is achieved by the processive behavior of the most abundant replicative DNA polymerases δ and ε which perform DNA synthesis of the six billion nucleotides that constitute the human genome. However, nature needs more flexibility. Indeed, when the replication complex encounters DNA distortions or persistent base modifications generated by endogenous or environmental insults, it frequently stalls because high selectivity of the replicative DNA polymerases means they are unable to faithfully insert a base opposite most lesions. To avoid an aberrant cessation of the cell cycle caused by the blockage of DNA replication, the stalled DNA polymerase needs to be transiently replaced by another DNA polymerase that is capable of bypassing the lesion. Because such lesion-bypass enzymes do not have a proofreading function, translesion synthesis (TLS) is inevitably accompanied by mutations at a high frequency that depends on the type of DNA damage and the particular polymerase(s) involved. In the course of evolution, multiple DNA polymerases have appeared to tolerate different types of DNA damage. Besides repair and recombination pathways which permit the removal or avoidance of DNA damage that would otherwise create mutations, this TLS tolerance pathway has developed from archaeal bacteria to humans [16]. The past decade have seen dramatic progress in our understanding of the world of eukaryotic DNA polymerases, especially whose involved in TLS. Human cells are now known to contain, besides Polδ and Polε, at least 10 additional nuclear DNA template-dependent DNA polymerases, named "error-prone" or "specialized" or "alternative", which could be categorized based on their infidelity in replicating undamaged DNA and their ability to contribute to DNA transactions in response to genotoxic stresses. Indeed, when the conformation of the double helix is regular and unbroken, Polδ and Polε copy DNA accurately. In contrast, when distortions or adducts disturb the DNA structure, some of these specialized DNA polymerases, called TLS DNA polymerases, including Polsζ, η, ι and κ take part in the replication of DNA damage that otherwise would not be tolerated [17,18]. This translesion synthesis past DNA lesions may be more or less accurate, depending on the DNA polymerase involved and the type of damage bypassed. However, in most cases the translesion process mediated by these specialized DNA polymerases is mutagenic. Therefore, the cell lifespan depends on a subtle equilibrium between accurate genomic DNA synthesis, necessary for the duplication of the genotype before chromosomal partition during mitosis, and less stringent DNA transactions involving the TLS DNA polymerases which allow cells to tolerate structural DNA perturbations, a necessary "flexibility" process.

3. Specialized DNA polymerases and translesion synthesis

Organisms from bacteria to man have long been known to contain more than one DNA polymerase. Over the past 50 years there has been a progressive accumulation of evidence for five 'classical' DNA polymerases in all mammalian cells, each functioning in DNA replication and/or repair (Polsα, β, δ, ε, γ). Polα, the first mammalian DNA polymerase identified, catalyses initiation of chromosomal DNA replication at origins and at Okazaki fragments on the lagging strand [19]; Polβ participates in base-excision repair [20]; Polγ performs mitochondrial DNA replication and repair [21]; Polδ participates in lagging-strand synthesis [22]; and Polε has a role in the synthesis of the leading strand of chromosomal DNA [23]. Ten additional DNA template-dependent DNA polymerases (see Table 1), each having a Greek letter designation (except Rev1) have been discovered in the last few years, all of which are able to perform translesion synthesis (TLS). The concept of TLS emerged with the discovery of the DNA polymerase characteristics of *Escherichia coli* DinB (Pol IV) and $UmuD_2'C$ (Pol V) proteins, which were known for decades as part as the "SOS" system involved in the error-prone response induced after genotoxic treatments [24]. Originally Din B was identified as the product of a gene involved in the *untargeted* mutagenesis on bacteriophage lambda in infected UV-irradiated *E. coli* bacteria. The *DIN B* gene is conserved in many bacteria as well as in eukaryotes, exception being *Saccharomyces cerevisiae* and *Drosophila melanogaster*. Din B belongs to the Y-family of TLS DNA polymerases which also includes the human DNA polymerases Polκ, ι, η and Rev 1. These enzymes share multiple common motifs in their primary sequences that are distinct from those of the previously known A-, B-, C-, and X-families of DNA polymerases. Nevertheless, they retain tertiary structures conserved in most DNA polymerases, i.e. right-handed architecture with fingers, palm, and thumb subdomains. Interest in this Y-family increased with the discovery that mutations in the POLH gene that encodes Polη are responsible for the variant form of xeroderma pigmentosum (XP-V), a rare autosomal recessive disorder characterized, as are the other forms of 'classical' XP,