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Review

DNA replication fidelity and cancer

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ABSTRACT

Cancer is fueled by mutations and driven by adaptive selection. Normal cells avoid deleterious mutations by replicating their genomes with extraordinary accuracy. Here we review the pathways governing DNA replication fidelity and discuss evidence implicating replication errors (*p*oint mutation *in*stability or PIN) in carcinogenesis.

1. Genetic instability and cancer

Tumor development is a multistep process requiring the accumulation of mutations that activate oncogenes or inactivate tumor suppressors [1–3]. To maintain normal cell functions, genetic stability is strictly controlled [4]. Therefore it is argued that defects in pathways governing genetic stability will facilitate tumorigenesis by fueling the reiterative process of mutation, selection and clonal expansion that drives cancer progression (reviewed and debated in [5–13]).

There is considerable evidence that genetic instability plays a role in cancer (reviewed in [3,13–16]). Cancer cells exhibit high frequencies of chromosomal aberrations [17,18], and the rates of gene rearrangements and amplifications are increased in many tumor cell lines [19–21]. Moreover, inherited deficiencies in genome maintenance systems contribute to human cancer susceptibility syndromes [22–24]. Defects in genes required for nucleotide excision repair (NER; Xeroderma pigmentosum), double-strand break recognition and repair (Ataxia telangiectasia, Nijmegen breakage syndrome), genetic recombination (Bloom, Werner and Rothmund–Thomson syndromes and BRCA1/2) and mismatch repair (MMR; Lynch syndrome) all cause genetic instability and are associated with human cancer syndromes. Thus, it is well established that chromosome instability (CIN) and microsatellite instability (MIN or MSI) predispose to cancer. Recently, sporadic cancers were shown to have elevated frequencies of random nucleotide point mutations, thus implicating point mutation instability (PIN) in oncogenesis [25,26].

Perhaps the strongest early evidence that increased spontaneous mutation (*i.e.*, mutator phenotype) contributes to human cancer was the discovery that defective mismatch repair (MMR) causes hereditary colon cancer (reviewed in [27–31]). In the early 1990s, colorectal cancer samples from Lynch syndrome pedigrees (also called hereditary nonpolyposis colon cancer or HNPCC) were noted to have microsatellite instability, normal cytogenetics, and were associated with a unique clinical presentation. Two groups simultaneously reported that these families carried mutations in *MSH2*, the gene encoding one of the primary proteins required for MMR [32,33]. Shortly thereafter, *MLH1*, the gene encoding another essential MMR protein, was cloned and found to be mutated in additional Lynch syndrome families [34,35]. The majority of Lynch syndrome patients inherit a mutation in either *MSH2* or *MLH1*, with a smaller percentage inheriting mutations in *PMS2* or *MSH6* [36,37]. The wild-type allele is then lost in tumors through LOH or gene silencing. Patients with inherited MMR deficiency not only are primarily susceptible to early-onset colorectal cancer, but also have an increased risk for extra-intestinal neoplasms. Inherited MMR defects are only responsible for a small number (1–5%) of colorectal cancer cases; thus, most colorectal cancers with MSI (~15% of all colorectal cancer cases) result from acquired defects in MMR, almost exclusively due to *MLH1* promoter hypermethylation [38]. MMR defects and MSI are also detected in non-colonic sporadic tumors, most commonly in endometrial, lung and gastric cancer [38,39].

MMR-deficient human tumor cell lines display increased spontaneous mutation rates with a preference for frameshifts and base substitution mutations [40–43]. Accordingly, microsatellite instability is a hallmark of MMR loss [31]. Microsatellite instability may be particularly relevant for colorectal cancer as many genes involved in intestinal carcinogenesis (*TGFβR2*, *APC*, *KRAS*, *BRAF*, and others) have repetitive DNA in their coding regions [44]. MSH6 defects are a less common cause of Lynch syndrome and result in predominantly extra-colonic tumors [45–49]. Interestingly, cells

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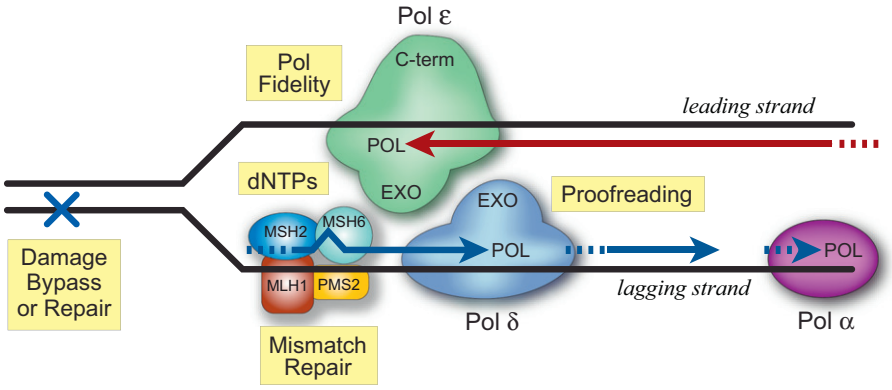

Fig. 1. Determinants of DNA replication fidelity. Schematic of a DNA replication fork with Pol ε and Pol δ on the leading- and lagging-strands, respectively. Major determinants of faithful DNA synthesis are highlighted in yellow. The polymerase domains (POL) of Pols ε and δ discriminate between correct and incorrect dNTPs prior to phosphodiester bond formation. If an error occurs, these are corrected primarily by the intrinsic proofreading exonuclease (EXO) present in each polymerase. Errors that escape proofreading are rectified by mismatch repair (MMR), which acts on both lagging (shown here) and leading (not shown) strands. DNA damage repair and dNTP pool ratios also influence replication fidelity.

defective for MSH6 have elevated rates of base-substitution mutations and lower levels of frameshifts, due to selective inactivation of MutSα [43,50,51]. Thus, intestinal carcinogenesis in Lynch syndrome may be directly related to MSI, whereas extra-colonic tumors may result from elevated base substitutions.

2. Determinants of DNA replication fidelity

Normal cells replicate their DNA with extraordinary fidelity ($\sim 10^{-10}$ mutations per base pair per cell division) [52]. This is achieved through the combined actions of polymerase base selectivity, $3' \rightarrow 5'$ exonucleolytic proofreading, mismatch correction and DNA damage repair (Fig. 1; reviewed in [53–71]).

Proofreading and MMR both contribute substantially to the overall fidelity of cellular DNA replication and mutation avoidance. Genetic experiments in *Escherichia coli* [53,54] and *Saccharomyces cerevisiae* [50,51,72–76] show that loss of either proofreading or MMR results in a 10–1000-fold increase in spontaneous mutation rate. Although studies in mammalian cells are more limited, cell-free fidelity assays [77,78] and experiments with MMR-deficient [40–43,79,80] and proofreading-deficient [81–84] cells also point to these repair pathways as major determinants of replication fidelity in higher eukaryotes. The prevailing model (Figs. 1 and 2) is that spontaneous errors by the replicative lagging- and leading-strand DNA polymerases (Pols δ and ε, respectively [85–87]) trigger proofreading by their intrinsic 3′-exonucleases. Occasional errors escape proofreading, and these are corrected by the MMR machinery. It is estimated that replicative eukaryotic DNA polymerases make errors approximately once every 10^4–10^5 nucleotides polymerized [58,59]. Thus, each time a diploid mammalian cell replicates, at least 100,000 and up to 1,000,000 polymerase errors occur.[1] The majority of these are base·base mispairs and ±1 slippage events [58,59], which must be corrected with almost 100% efficiency to achieve a spontaneous mutation rate of $\sim 10^{-10}$ per base pair per cell division [52].

Repair of promutagenic DNA damage (both spontaneous and induced [22,68–71]) and maintenance of normal dNTP pools [88–90] are also important determinants of replication fidelity. Similar to proofreading and mismatch repair, defects in individual enzymes affecting dNTP pool ratios confer spontaneous mutator phenotypes [88–90]. In contrast, most single-gene defects in DNA damage repair pathways exhibit near-normal spontaneous mutation rates and reveal themselves as "conditional mutators" when cells are challenged with DNA damaging agents [71]. One exception is the repair of 8-oxo-G lesions by the MutM/MutY/MutT "GO" system [54,91–95]. In *E. coli*, loss of either *MutM* or *MutT* confers a moderate-to-strong mutator phenotype in the absence of exogenous oxidative stress [54,92,96]. However, defects in homologous mouse genes have only modest effects on spontaneous mutation rates, presumably due to different interactions of redundant pathways that prevent or repair oxidative DNA damage in mammals [71,93–95].

Quantitative estimates of spontaneous DNA degradation in cells suggest that the daily dose of promutagenic damage is substantial [97–99] (Fig. 3A). These lesions result from the intrinsic chemical instability of DNA under physiological conditions and the exposure of DNA to active oxygen and other reactive metabolites and coenzymes that are generated by normal cells [97,99]. Altogether, it is estimated that ~20,000 potentially mutagenic lesions arise per diploid mammalian cell per day. Most of these lesions are repaired by the base excision repair (BER) pathway [68,69]. This repair must occur efficiently prior to DNA replication for cells to maintain a low spontaneous mutation rate. The toll of 20,000 spontaneous lesions per cell per day is high, and this is in addition to the 100,000–1,000,000 DNA polymerase errors that occur in replicating cells over the same approximate time frame (Fig. 3B).

Thus, there are a number of repair systems that ensure faithful DNA replication. Among these, polymerase proofreading and MMR play primary roles as evidenced by the strong mutator phenotypes conferred by loss of either pathway in the absence of exogenous DNA damage.

2.1. Proofreading polymerases

There are 3 eukaryotic DNA polymerases that have intrinsic $3' \rightarrow 5'$ exonucleolytic proofreading activity: Pol δ, Pol ε and Pol γ [60,100]. Only Pols δ and ε are nuclear, while Pol γ is mitochondrial. Similar to other proofreading DNA polymerases, Pols δ and ε are comprised of multiple subunits with the catalytic peptide of each enzyme harboring both polymerase (pol) and exonucle-

[1] Calculated as follows: the mammalian diploid genome has 6×10^9 base pairs, which corresponds to 1.2×10^{10} nucleotides that must be polymerized each time a cell divides. DNA polymerases δ and ε replicate the bulk of genomic DNA and have error rates of 10^{-4} to 10^{-5} per nucleotide polymerized (prior to correction by proofreading or MMR). Thus, if the error rate is 10^{-5}, then 10^{10} nucleotides per genome $\times$ 10^{-5} errors per nucleotide = 100,000 polymerase errors per genome per cell division. If the error rate is 10^{-4}, then 10^{10} nucleotides per genome $\times$ 10^{-4} errors per nucleotide = 1,000,000 errors per genome per cell division.