Review Article

Translesion Synthesis Polymerases in the Prevention and Promotion of Carcinogenesis

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A critical step in the transformation of cells to the malignant state of cancer is the induction of mutations in the DNA of cells damaged by genotoxic agents. Translesion DNA synthesis (TLS) is the process by which cells copy DNA containing unrepaired damage that blocks progression of the replication fork. The DNA polymerases that catalyze TLS in mammals have been the topic of intense investigation over the last decade. DNA polymerase η (Pol η) is best understood and is active in error-free bypass of UV-induced DNA damage. The other TLS polymerases (Pol ι, Pol κ, REV1, and Pol ζ) have been studied extensively *in vitro*, but their *in vivo* role is only now being investigated using knockout mouse models of carcinogenesis. This paper will focus on the studies of mice and humans with altered expression of TLS polymerases and the effects on cancer induced by environmental agents.

1. Introduction

Tumorigenesis is a multistep process beginning with the transformation of a single cell by the accumulation of at least six distinct characteristics. These include infinite lifespan, resistance to antigrowth signals, resistance to apoptosis, autocrine production of growth signals, sustained angiogenesis, and tissue invasion [1]. Most environmental carcinogens induce transformation by causing mutations in the DNA that alter the activity of protooncogenes or tumor suppressors. These mutations are formed when residual, unrepaired DNA damage stalls progression of the replication fork during S phase. Stalled replication forks are most frequently resolved using error-free mechanisms that include homologous recombination or use of the homologous nascent strand as a template. Nevertheless, replication may proceed using the damaged strand as a template in an error-prone process known as translesion DNA synthesis (TLS). TLS is defined as the incorporation of a nucleotide across from DNA damage followed by extension of the potentially mispaired primer-template, and can be error-free or error-prone. Cellular commitment to error-free, recombinatorial damage avoidance or error-prone TLS is modulated by the molecular switch PCNA (Figure 1). Cells presumably risk mutations caused by TLS to relieve replication fork blockage at DNA adducts and to avoid the potential formation of extremely cytotoxic double strand breaks (DSB). Although it accounts for less than 10% of all bypass synthesis events in yeast [2], the frequency of potentially mutagenic TLS may be as high as 50% in higher eukaryotes [3–5]. The propensity and mutagenic potential of TLS explain why it is etiologic in most environmentally-induced cancers and has been the focus of numerous investigations over the past decade.

TLS is performed by a relatively new category of accessory DNA polymerases. Polymerase η (Pol η), Pol ι, Pol κ, and REV1 in the Y-family [6] and Pol ζ in the B-family [7, 8] are responsible for most TLS in mammalian cells. These proteins have active sites that are larger and more open than those of the high-fidelity replicative DNA polymerases (Pol α, δ, and ε), allowing accommodation of and synthesis past

DNA templates with large, helix-distorting lesions [9]. This unique ability to synthesize DNA opposite bulky adducts helps cells avoid double strand breaks associated with replication fork stalling, but can also lead to mutagenesis by incorrect base addition. It is important to note that polymerases in the Y-family are expressed in all three kingdoms of life, indicating a critical and evolutionarily conserved role for these proteins [6]. The obviously conflicting roles of these enzymes in both preventing and promoting genetic instability are reflected in the tight cellular control of the TLS pathway (Figure 1). Although extensive *in vitro* studies have given us a better understanding of their role in the cell, much less is known about the function of TLS polymerases in living animals. Limited epidemiological studies have been conducted to associate single nucleotide polymorphisms (SNPs) with cancer risk in humans. Knockout mice have been generated for each gene, and carcinogenesis studies are published or underway. Importantly, studies in mice and humans have shown that TLS polymerases, particularly Pol η, are involved in immunoglobulin gene hypermutation. Readers are directed to reviews by Reynaud et al. and Diaz et al. for an exploration of this function of TLS polymerases [10, 11]. This review will focus on the rapidly progressing connection of TLS and cancer research in knockout mice and human populations.

2. REV1

REV1 was discovered in budding yeast by the Lawrence group in 1989 as a component of the Pol ζ complex [17]. The catalytic activity of REV1 is limited to insertion of dCMP across from a template dG [18]. The human homolog was cloned in 1999 and has the same template-dependent dCMP transferase catalytic activity on an undamaged template or an abasic site [19]. One locus used in eukaryotic cells to measure mutation frequency is *HPRT*, a gene involved in the purine salvage pathway. In this forward mutation assay, cells with loss-of-function mutations in *HPRT* are resistant to the drug 6-thioguanine (TG). REV1 is required for carcinogen-induced *HPRT* mutagenesis in human cells [20–22], but the catalytic activity appears to be dispensable for the induction of UV-induced mutations [23, 24], indicating that this protein probably plays a structural rather than catalytic role in UV mutagenesis.

Cells from mice with a targeted deletion of the BRCA1 C-terminal (BRCT) homology domain of Rev1 (*Rev1*$^{B/B}$) have a reduced UV-induced mutation frequency at the *Hprt* locus [25]. However, the animals have a paradoxically decreased latency of squamous cell carcinoma (SCC) formation and only marginally reduced p53 mutagenesis in the skin after UV exposure [26]. Despite *Rev1*$^{B/B}$ cells showing a moderate increase in chromatid breaks and exchanges after UV *in vitro* [25], comparative genomic hybriziation of UV-induced SCC and normal skin DNA reveals no increase in the frequency of gross genomic alterations in *Rev1*$^{B/B}$ SCC [26]. If point mutations and chromosomal rearrangements are near normal levels in BRCT-deleted *Rev1*$^{B/B}$ mice, what is the reason for accelerated SCC development? Acute UV exposure
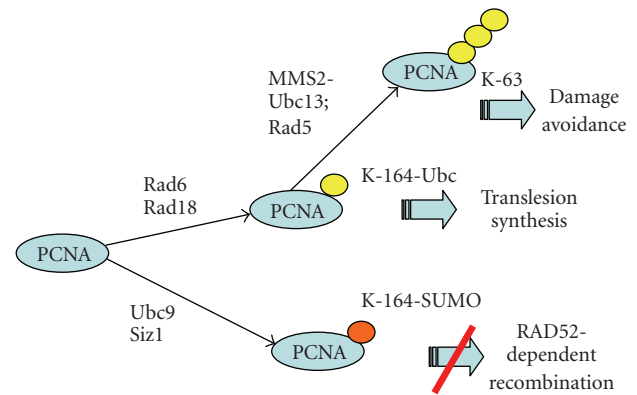

Figure 1: Regulation of DNA lesion bypass in *Saccharomyces cerevisiae* and humans. Bulky DNA lesions can cause blockage of replicative polymerases and replication fork stalling. The ubiquitin conjugase/ubiquitin ligase pair Rad6/Rad18 is recruited to stalled replication forks where the proteins catalyze monoubiquitylation of PCNA at lysine 164. TLS proteins such as REV1 and Pol η have increased affinity for monoubiquitylated PCNA, which facilitates their recruitment and the completion of TLS. In yeast, Rad5 and the MMS2-Ubc13 complex (UBE2V2-UBE2N in humans) can catalyze polyubiquitylation of PCNA via lysine 63 of ubiquitin, which blocks TLS and activates error-free damage avoidance. Damage avoidance includes template switching, during which the nascent DNA strand from the sister duplex is used as an undamaged homologous template to replicate past the lesion. Humans express two Rad5 homologs, SHPRH and HLTF, and both catalyze K-63-linked polyubiquitylation of PCNA in human cells [12–15]. In yeast, Ubc9-Siz1 can attach the small ubiquitin-like modifier (SUMO) to lysine 164 of PCNA in a reaction that competes with Rad6/Rad18-mediated monoubiquitylation. PCNA SUMOylation at K-164 attracts the helicase Srs2 and prevents error-prone RAD52-dependent recombination. Reproduced with permission from Watson et al. [16].

of these *Rev1*-mutant mice induces enhanced Atr signaling, senescence, and apoptosis in the skin. However, long-term low-dose UV exposure causes a mitogenic response, as evidenced by epidermal hyperplasia, decreased apoptosis, and increased proliferation of CPD-containing keratinocytes [26]. Based on literature reports of the etiological role of IL-6 in carcinogenesis and elevated IL-6 levels in the skin after a single subtoxic UV dose, the authors conclude that error-prone TLS of UV-induced DNA damage is responsible for suppressing the proinflammatory, tumor-promoting effects of UV in the skin. However, more direct immunological studies are needed to confirm that Rev1 suppresses UV-induced inflammation and tumor suppression.

REV1 has also been implicated in TLS across other types of DNA lesions. Benzo[a]pyrenediolepoxide (BPDE) is the primary carcinogenic metabolite of B[a]P and causes point mutations in a REV1-dependent manner [22, 27]. BPDE-induced *Hprt* mutations are dramatically decreased in primary mouse fibroblasts after ribozyme-mediated *Rev1*-knockdown. When a plasmid expressing this ribozyme is delivered to the lungs of A/J mice by aerosol nebulization,

Rev1 mRNA is reduced by ∼ %50 in the bronchial epithelium. This targeted gene therapy causes a ∼ %40 reduction in the lung tumor multiplicity after B[a]P treatment. In addition, only 73% of ribozyme-treated mice develop lung adenomas after B[a]P, compared with 100% penetrance in control animals [28]. This report highlights the potential for interrupting translesion synthesis as a chemoprevention strategy.

Although no human disorder involving *REV1* deficiency is known, there are 16 SNPs in humans that result in nonsynonymous amino acid changes. The F257S SNP, which lies outside of all known functional domains of the protein, has been associated with an increased risk of squamous cell carcinoma of the lung in patients who have ever smoked cigarettes [29]. However, this association remains controversial [30]. The same F257S SNP was associated with decreased risk of cervical cancer, and N373S within the catalytic domain was associated with increased risk of cervical cancer. Both effects were specific for squamous cell carcinoma and not relevant for adenocarcinoma of the cervix [31]. Although the functional consequences of these polymorphisms are unknown, these studies support a role for REV1 in the formation of multiple internal cancers.

3. Pol η

The study of translesion synthesis in mammals began in 1999 with the discovery of the molecular defect that results in Xeroderma Pigmentosum (XP) variant syndrome. All XP patients have dramatically increased susceptibility to UV-induced skin cancer [32]. Patients in complementation groups A through G are deficient in nucleotide excision repair (NER), the major pathway for removal of helix-distorting lesions, including those induced by UV. However, the XP variant subset of patients has normal NER activity [33, 34], yet displays the skin cancer-prone phenotype of NER-deficient patients. The XP variant mystery persisted for nearly three decades. Intensive investigations indicate that after UV-irradiation cells from these patients have difficulty exiting S-phase that is exacerbated by caffeine [35, 36]. Further, these cells are extremely hypermutable after UV [37]. In 1999, two groups independently discovered that XP variant patients carry autosomal recessive mutations in *POLH*, the human gene coding for Pol η, and that the enzyme can catalyze error-free DNA synthesis across from a template TT cyclobutane pyrimidine dimer (CPD) [38–40]. The dramatic increase in skin cancer risk of XP variant patients could now be explained by the absence of a critical translesion DNA polymerase. UV principally induces photoaddition products between intrastrand adjacent pyrimidines, the most frequent of which are TT CPD. These lesions block progression of the replication fork. Data indicate that helicase activity may continue in spite of the blocked replication complex, resulting in single-stranded DNA that is rapidly coated with replication protein A (RPA). This appears to attract the ubiquitin ligase RAD18, which has binding sites for the ubiquitin conjugase RAD6, Pol η, and RPA. One target of ubiquitylation is PCNA. Since Pol η has a ubiquitin binding domain, Pol η is now thought to be preferentially attracted to the stalled fork because it is chaperoned directly by RAD18 and binds to the ubiquitylated PCNA (Figure 1) [41]. Data indicate that Pol η then incorporates AA across from TT CPD in the template. In the absence of Pol η, another translesion polymerase, which is potentially error-prone when bypassing these common UV-induced lesions, accesses the damaged template (reviewed in [42, 43]). Generation of Pol η-knockout mice shows that the highly homologous mouse Pol η protein functions similarly in UV-induced mutagenesis and carcinogenesis. Pol η-deficient mice develop squamous cell carcinoma with 100% penetrance at a UV fluence that does not cause any tumors in wild-type littermates. In addition, approximately one-third of heterozygous mice develop cancer after UV exposure [44]. This raises the possibility that humans carrying heterozygous mutations in the *POLH* gene may have an increased risk of developing skin cancer. However, this speculation has not been clinically investigated.

There is evidence that XP variant patients develop internal cancers faster than Pol η-proficient individuals [45, 46], raising the possibility that Pol η-deficiency is involved in the formation of multiple human cancers caused by DNA damaging agents other than UV. Six SNPs in *POLH* have been found to date that result in nonsynonymous amino acid substitutions, but their functional significance is unknown. There is a single study evaluating the effects on cancer risk of *POLH* polymorphisms. Flanagan and colleagues found no significant changes in coding-region SNPs of *POLH* among 40 basal cell carcinoma and squamous cell carcinoma patients in a fair-skinned Irish population [47]. It is clear that larger epidemiological studies of *POLH* status are needed to evaluate the effects of *POLH* polymorphisms.

4. Pol ι

DNA polymerase ι (Pol ι) was discovered in 1999 as a novel homolog of Pol η in mammals and is encoded by the human *POLI* gene [48]. *In vitro* studies with purified enzyme indicate error-prone TLS function on almost all substrates examined, perhaps due to the still controversial ability of Pol ι to incorporate incoming nucleotides using Hoogsteen base pairing [49, 50]. Exhaustive characterization of the error-prone replication properties of Pol ι has lent credibility to the hypothesis that *Poli* is a candidate gene for the Pulmonary adenoma resistance 2 (*Par2*) locus in mice [51–53]. The *Par2* locus was identified in 1996 by chromosomal linkage mapping between BALB/cJ and A/J mouse strains and plays a major role in the relative resistance of BALB mice versus the A/J strain to developing urethane-induced lung adenomas [54]. Wang et al. identified ten amino acid-substitution polymorphisms between A/J and BALB mice that produce changes in substrate recognition of Pol ι; while the enzyme from both strains is functional, the isoform expressed in BALB mice may be more accurate on certain undamaged templates [52]. These studies

suggest that Pol ι acts to suppress urethane-induced lung adenomas. It has been hypothesized that this activity is due to the augmentation of base excision repair (BER) by Pol ι, because the enzyme has $5'$ deoxyribose phosphate (dRP) lyase activity and can partially reconstitute the BER-deficiency of Pol β-null cells *in vitro* [55]. It is possible that after urethane-induced DNA damage, which produces 1, N^6-ethenoadenine adducts [56] that are primarily repaired by BER [57], Pol ι acts in the gap-filling step of lesion repair. If the isoform of Pol ι expressed in A/J mice is more likely to add the incorrect G opposite a template T in the gap-filling step of BER, as was found *in vitro* [52], this could explain the increased incidence of lung adenomas in A/J mice. In support of this hypothesis, nearly all urethane-induced adenomas in mice have a CAA → CGA transition in codon 61 of *Kras2* [51]. In addition, 129-derived mouse strains that carry a SNP in codon 27 of *Poli* resulting in a severely truncated protein [58] display extreme sensitivity to urethane-induced lung adenomas [53]. In the absence of Pol ι, it has been hypothesized that another DNA polymerase, such as Pol β, inserts the incorrect base during gap filling in the repair of urethane-induced DNA damage. However, normal mouse Pol ι displays extremely error-prone properties during synthesis opposite all four undamaged template bases *in vitro* [58], making it unlikely to prevent mutations during BER in mice that are Pol ι-competent. Further studies must be completed to determine the tumor suppression mechanism of Pol ι in mouse lung carcinogenesis.

A growing body of evidence suggests that Pol ι is involved in error-prone TLS of UV-induced DNA damage *in vivo*. The heightened UV mutagenesis of Pol η-null (XP variant) human cells has been attributed to TLS by Pol ι [59]. Loss of the functional *Poli* gene in dermal cells results in a dramatically reduced UV-induced mutation frequency at the *Hprt* locus in both wild-type and Pol η-deficient mice (Figure 2). Remarkably, however, the decreased UV-induced mutagenesis observed due to loss of the error-prone Pol ι from Pol η-deficient mice is associated with *increased* cancer risk after UV exposure (Figure 3) [60]. This result was confirmed and extended by Ohkumo and colleagues who showed that *Poli*$^{-/-}$ mice are more likely to develop aggressive mesenchymal tumors after UV than *Poli*-proficient siblings [61]. These apparently contradictory findings speak to the fact that cancer etiology is more complex than the point mutations scored by the *Hprt* assay, and that one cannot use cell biology alone to accurately predict cancer risk in a TLS model. Indeed, they suggest a tumor suppressor role for Pol ι that could be separate from its role as a TLS polymerase prone to induce single base-substitution mutations. It is also possible that Pol ι is error-free when bypassing a minor UV adduct, or that it is involved in error-free BER of the minimal oxidative damage induced by UVB used in these studies [62], but more detailed experiments must be performed to evaluate these possibilities.

The role of Pol ι in the induction of cancer induced by other carcinogens has not been systematically studied to date. It is interesting to note that Newcomb et al. found
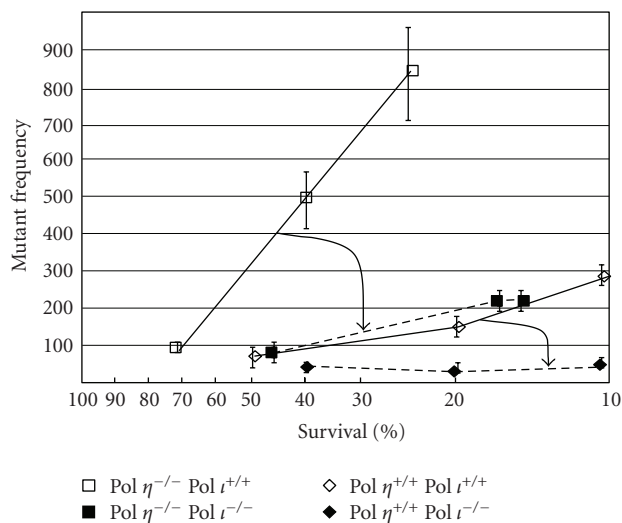

FIGURE 2: Frequency of 6-thioguanine-resistant (TG^r) clones as a function of survival after UV irradiation. Cells were plated on three 150-mm-diameter dishes at a density of 10^4 cm^{-2} to determine mutant frequency or at cloning density to determine survival. After attachment, plates were irradiated with UV fluences to yield 20%–40% survival. The actual survival in the mutagenesis experiments was determined by refeeding the survival plates at one week and staining with crystal violet after two weeks. Percent survival for each UV fluence was corrected for replating and plotted on the x-axis. The corresponding mutant frequency at each survival is plotted on the y-axis. Each point represents the mean of three independent dishes at the indicated survival, ± 1 SD. Mutant frequency at the *Hprt* locus is defined as the number of TG^r clones per million clonable cells. Each data point represents independent experiments in which 2-4 × 10^6 surviving cells were selected after UV irradiation and an 8- to 9-day expression period. The data have been corrected for cloning efficiency on the day of selection, and the spontaneous background mutant frequency (1×10^{-5}) has been subtracted. The arrows indicate the reduction in mutant frequency when Polι is disrupted in the Pol η-deficient background (larger arrow) and in the Pol η-proficient background (smaller arrow). Reproduced with permission from Dumstorf et al. [60].

that Pol ι-deficient 129 mice are resistant to γ-irradiation-induced thymic lymphoma but sensitive to methylating agent-induced thymic lymphoma [63]. γ-Irradiation induces DNA strand breaks and oxidative damage, and Pol ι is known to protect cells from oxidative stress [64]. It is therefore possible that increased cell death after γ-irradiation protects *Poli*$^{-/-}$ 129 mice from lymphomagenesis. However, Pol ι does not affect the sensitivity of Pol β-null cells to methylating agents [65], so the sensitivity of 129 mice to thymic lymphoma induced in this way is still unexplained.

There is no known human disorder involving deficiency for Pol ι. However, Pol ι is overexpressed in some lung cancer cell lines [52] as well as in primary human gliomas [66]. The T706A SNP was found to increase the risk of adenocarcinoma and squamous cell carcinoma of the lung in persons < 61 years of age [29]. However, this association was not confirmed by another independent study [67] and failed to show significance in a meta-analysis [68].
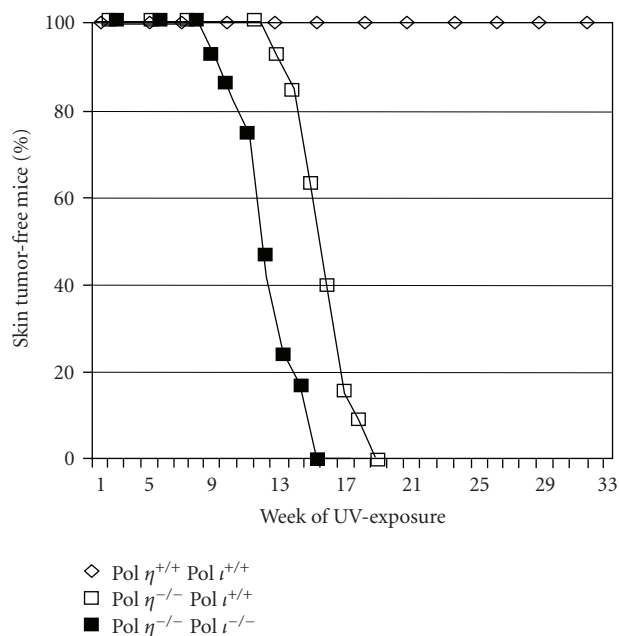
FIGURE 3: UV light-induced skin cancer in mice. Mice were shaved once per week and irradiated three times per week with 3.75 kJ/m^2 for 20 weeks or until the first skin tumor arose. Mice were inspected weekly for the development of skin tumors. All 12 homozygous *Polh* knockout mice (open diamonds) developed skin tumors by 18 weeks, while all 12 *Polh*$^{-/-}$*Poli*$^{-/-}$ mice (open circles) developed skin tumors by 13 weeks. This *Poli*-dependent decrease in tumor latency is highly significant ($P < .0002$). No difference was found in the histological analysis of skin tumors among the groups. Reproduced with permission from Dumstorf et al. [60].

Another SNP in human *POLI*, F532S, is associated with prostate cancer patients whose tumors display *TMPRSS2-ERG* fusion with a highly significant odds ratio of 4.6 [69]. The protooncogenic transcription factor *ERG* was identified as the most frequently overexpressed gene in human prostate cancers [70], and fusion with the androgen-responsive serine protease *TMPRSS2* by chromosomal rearrangement was found in >90% of *ERG*-overexpressing cases [71]. Threonine 706 and serine 532, the two residues altered by these SNPs in Pol ι, are located in the noncanonical ubiquitin-binding motifs UBM2 and UBM1, respectively [72]. These two polymorphisms could therefore affect binding of Pol ι to ubiquitylated PCNA, which is required for its recruitment to stalled replication forks following DNA damage. In the case of prostate cancer, the F532S variant of Pol ι may promote chromosomal instability by causing replication fork stalling and double-strand break (DSB) formation. DSB formed in this way could promote cellular transformation by causing chromosomal rearrangements that place the protooncogene *ERG* under control of the androgen-responsive promoter elements of *TMPRSS2* and lead to *ERG*-overexpression as is found in many prostate cancers [71]. Evidence supports the suppression of skin and lung cancers by Pol ι in humans and mice, and new studies suggest that other cancers could be

affected by this protein, making it a promising candidate for future investigation.

5. Pol κ

The fourth member of the Y-family is DNA polymerase κ. Pol κ performs faithful TLS of BPDE-induced DNA damage *in vitro* by inserting dC opposite a template BPDE-adducted G [73–75]. Pol κ is required for recovery from a novel BPDE-induced intra-S phase checkpoint, and the protein relocalizes to stalled replication forks after BPDE-induced DNA damage [76, 77]. *Polk*$^{-/-}$ mouse embryonic fibroblasts (MEFs) show persistent S-phase arrest after BPDE exposure, which results in increased DSB formation at stalled replication forks and increased toxicity in cells without functional Pol κ [77]. Avkin and colleagues measured TLS efficiency and fidelity in *Polk*$^{-/-}$ MEFs using a shuttle vector technique. TLS efficiency on a plasmid containing a site-specific BPDE-N^2-dG adduct is reduced nearly threefold in *Polk*$^{-/-}$ MEFs, and mutagenic TLS is increased from 29% to 50% in knockout cells, supporting a role for Pol κ in the efficient and error-free bypass of BPDE DNA damage [78]. siRNA-mediated *POLK*-knockdown also reduces the efficiency of TLS past BPDE-N^2-dG in human U2OS cells [79]. This body of evidence suggests that Pol κ could have an important role in cancers caused by bulky chemical carcinogens like BPDE. Pol κ has also recently been linked to nucleotide excision repair. *Polk*$^{-/-}$ MEFs have reduced levels of NER of UV damage, including reduced repair synthesis and removal of 6-4 photoproducts after UV. Both of these phenotypes are largely corrected by expressing wild-type Pol κ, but not a catalytically inactive mutant [80]. Pol κ carries out NER repair synthesis and is recruited to sites of NER through its interaction with XRCC1 and ubiquitylated PCNA [81]. These remarkable studies highlight the ability of TLS polymerases to function in multiple cellular pathways and the likelihood that *Polk* plays an important role in preventing DNA damage-induced carcinogenesis. While *Polk*-knockout mice have been generated [82, 83] and show increased spontaneous mutagenesis in kidney, liver, and lung [84], no cancer studies have yet been reported using these models.

Pol κ is overexpressed in ∼70% of nonsmall cell lung cancers (NSCLC) examined [85], and this overexpression correlates with mutation status of *TP53* [86] which is itself an indicator of poor prognosis [87]. In addition, *POLK* promoter activity is increased in *TP53*$^{-/-}$ cells, and p53 protein suppresses *POLK* promoter activity *in vitro*. These reports suggest that Pol κ overexpression in NSCLC could be secondary to loss of functional p53, but the correlation between these two events must be investigated to rule out an etiological role for Pol κ in lung cancer. Much stronger epidemiological evidence shows that Pol κ is overexpressed in gliomas. Multivariate analysis indicates that Pol κ overexpression is an independent prognostic factor for the assessment of glioma patient outcomes (Figure 4) [66]. Although the potential role of Pol κ in the etiology of brain tumors is unclear, Pol κ is clearly a candidate for
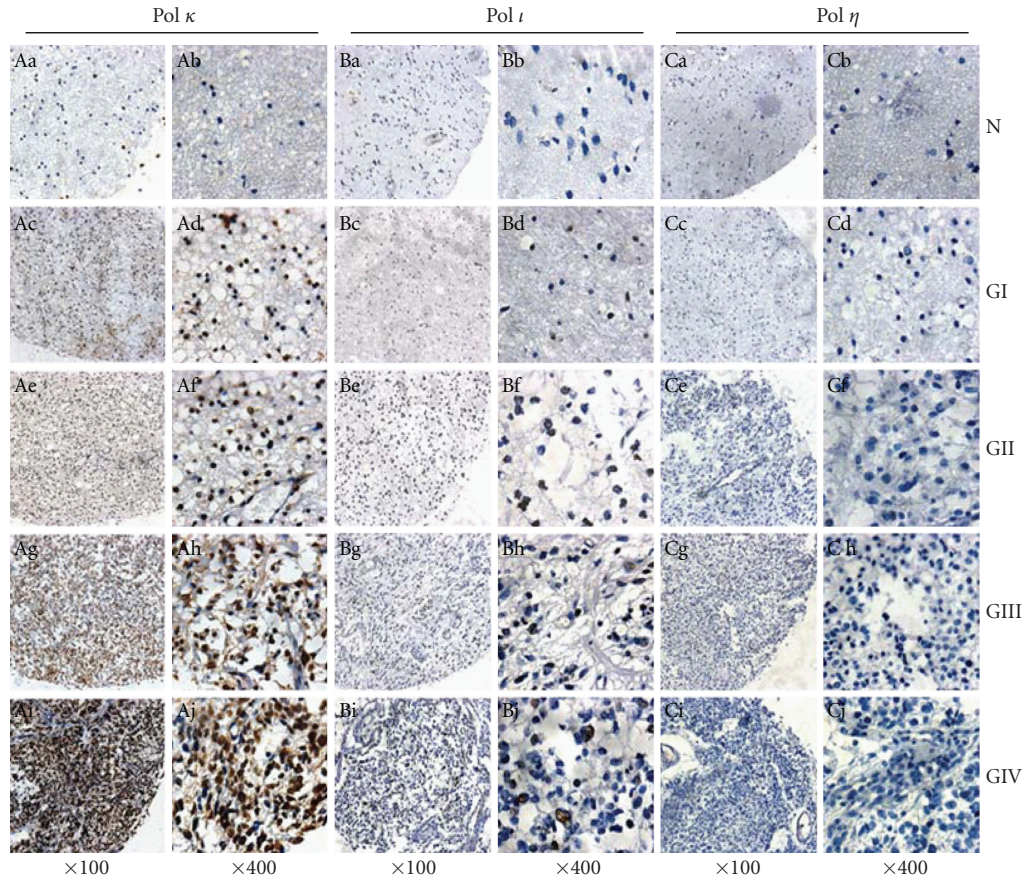
FIGURE 4: Immunohistochemical analysis of Pol κ, Pol ι, and Pol η expression in primary glioma tissues (g) and normal brain tissues (n). Paraffin-embedded tissue microarrays comprising 104 primary glioma specimens from WHO grades I-IV were stained for Pol κ, Pol ι, or Pol η. Representative images of Pol κ, Pol ι, and Pol η expression: Aa, Ab, Ba, Bb, Ca, and Cb, normal brain tissue; Ac, Ad, Bc, Bd, Cc, and Cd, pilocytic astrocytoma (WHO grade I); Ae, Af, Be, Bf, Ce, and Cf, diffuse astrocytoma (WHO grade II); Ag, Ah, Bg, Bh, Cg, and Ch, anaplastic astrocytomas (WHO grade III); Ai, Aj, Bi, Bj, Ci, and Cj, glioblastoma multiforme (WHO grade IV); magnification: X100 (Aa, Ac, Ae, Ag, Ai, Ba, Bc, Be, Bg, Bi, Ca, Cc, Ce, Cg, and Ci) and X400 (Ab, Ad, Af, Ah, Aj, Bb, Bd, Bf, Bh, Bj, Cb, Cd, Cf, Ch, and Cj). Reproduced with permission from Wang et al. [66].

investigation of cancer risk and chemoprevention of multiple tumor types.

6. Pol ζ

The human homolog of yeast DNA Polymerase ζ is required for mutagenesis by UV, BPDE, and other carcinogens [7, 88]. Pol ζ belongs to the B-family of DNA polymerases and contains a large catalytic subunit encoded by the *REV3* gene in humans [7] along with the much smaller regulatory protein REV7 [89]. Early investigations in *Saccharomyces cerevisiae* showed that rev3 mutant strains have reduced rates of spontaneous mutation [90], indicating that Pol ζ is involved in the mutagenic processing of spontaneous and UV-induced mutations. Studies in mammalian cells indicate that Pol ζ has a role in both repair of double strand breaks and base substitution mutagenesis, the latter likely involving extension of mispaired primer termini after initial TLS by another polymerase [91, 92]. Pol ζ is the only TLS polymerase required for development, and complete *Rev3*-knockout results in mitotic catastrophe and lethality at mouse embryonic day 10.5 [93–95]. However, conditional *Rev3*-knockout mice have been generated and are viable and fertile. While *Rev3*-deficiency alone is insufficient to promote cancer formation, conditional *Rev3* knockout accelerates the spontaneous formation of lymphoma in $Trp53^{-/-}$ mice. In humans, *REV3* gene expression is reduced by twofold in 40 of 74 (54%) colon carcinomas compared to matched normal tissue [96]. However, normal expression is found in much smaller sample sets of gastric, colon, lung, and renal cancers [97], and the gene is not mutated in primary tumors or cell lines from breast and colon cancers [89]. The expression levels of *REV3* in human cancers, particularly colon carcinoma, must be revisited using larger sample sizes to draw firm conclusions about the correlation of gene expression and cancer progression. No studies are published investigating the 25 nonsynonymous SNPS in human *REV3*, but the possibility exists that functional changes in human Pol ζ could alter the risk of cancer formation.

7. Conclusions

The importance of translesion DNA synthesis in preventing human cancer is well understood from the example of XP variant, in which patients lacking the Y-family DNA polymerase η are prone to develop UV-induced skin cancers due to an extremely hypermutable phenotype. However, we understand very little about how the other polymerases involved in TLS affect human health and cancer risk. Recently developed mouse models have so far provided conflicting results; ribozyme-mediated knockdown of total Rev1 and removal of the BRCT domain both result in reduced mutagenesis by BPDE or UV, respectively. As expected, when *Rev1* mRNA is knocked down using the same ribozyme delivered to the lungs of mice, multiplicity of B[a]P-induced lung adenomas decreases [28]. In contrast, Rev1 BRCT-null mice develop UV-induced squamous cell carcinomas *faster* than wild-type controls [26]. In a similarly paradoxical finding, mice lacking both Pol η and Pol ι have decreased UV-induced mutations in their dermal fibroblasts and accelerated development of squamous cell carcinoma after UV treatment compared to Pol ι-proficient animals [60]. While it is understood that the mutations induced by these polymerases are etiological in many environmentally-induced cancers, it is clear from these studies that simply blocking TLS is not sufficient to reduce cancer risk, and in fact may cause an acceleration of carcinogenesis. More detailed studies are needed using existing mouse models to determine the effects of TLS polymerase activity on cancer development after diverse carcinogen exposures. In addition, molecular epidemiological studies must be conducted to evaluate the functional consequences of the many nonsynonymous SNPs in TLS polymerase genes, some of which have already been associated with cancer risk or protection. After a decade of intense research, there are still critical gaps in our understanding of the role of TLS in human health and cancer risk.

Acknowledgments

References

[1] D. Hanahan and R. A. Weinberg, "The hallmarks of cancer," *Cell*, vol. 100, no. 1, pp. 57–70, 2000.

[2] K. Baynton, A. Bresson-Roy, and R. P. P. Fuchs, "Analysis of damage tolerance pathways in *Saccharomyces cerevisiae*: a requirement for Rev3 DNA polymerase in translesion synthesis," *Molecular and Cellular Biology*, vol. 18, no. 2, pp. 960–966, 1998.

[3] S. Adar, L. Izhar, A. Hendel, N. Geacintov, and Z. Livneh, "Repair of gaps opposite lesions by homologous recombination in mammalian cells," *Nucleic Acids Research*, vol. 37, no. 17, pp. 5737–5748, 2009.

[4] K. M. Vasquez, K. Marburger, Z. Intody, and J. H. Wilson, "Manipulating the mammalian genome by homologous recombination," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 15, pp. 8403–8410, 2001.

[5] M. J. Shulman, C. Collins, A. Connor, L. R. Read, and M. D. Baker, "Interchromosomal recombination is suppressed in mammalian somatic cells," *EMBO Journal*, vol. 14, no. 16, pp. 4102–4107, 1995.

[6] H. Ohmori, E. C. Friedberg, R. P.P. Fuchs et al., "The Y-family of DNA polymerases," *Molecular Cell*, vol. 8, no. 1, pp. 7–8, 2001.

[7] P. E. M. Gibbs, W. G. McGregor, V. M. Maher, P. Nisson, and C. W. Lawrence, "A human homolog of the *Saccharomyces cerevisiae* REV3 gene, which encodes the catalytic subunit of DNA polymerase ζ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 12, pp. 6876–6880, 1998.

[8] W. Lin, X. Wu, and Z. Wang, "A full-length cDNA of hREV3 is predicted to encode DNA polymerase ζ for damage-induced mutagenesis in humans," *Mutation Research*, vol. 433, no. 2, pp. 89–98, 1999.

[9] S. D. McCulloch and T. A. Kunkel, "The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases," *Cell Research*, vol. 18, no. 1, pp. 148–161, 2008.

[10] C.-A. Reynaud, F. Delbos, A. Faili, Q. Gueranger, S. Aoufouchi, and J.-C. Weill, "Competitive repair pathways in immunoglobulin gene hypermutation," *Philosophical Transactions of the Royal Society B*, vol. 364, no. 1517, pp. 613–619, 2009.

[11] M. Diaz and C. Lawrence, "An update on the role of translesion synthesis DNA polymerases in Ig hypermutation," *Trends in Immunology*, vol. 26, no. 4, pp. 215–220, 2005.

[12] A. Motegi, H.-J. Liaw, K.-Y. Lee et al., "Polyubiquitination of proliferating cell nuclear antigen by HLTF and SHPRH prevents genomic instability from stalled replication forks," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 34, pp. 12411–12416, 2008.

[13] A. Motegi, R. Sood, H. Moinova, S. D. Markowitz, P. P. Liu, and K. Myung, "Human SHPRH suppresses genomic instability through proliferating cell nuclear antigen polyubiquitination," *Journal of Cell Biology*, vol. 175, no. 5, pp. 703–708, 2006.

[14] I. Unk, I. Hajdú, K. Fátyol et al., "Human HLTF functions as a ubiquitin ligase for proliferating cell nuclear antigen polyubiquitination," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 10, pp. 3768–3773, 2008.

[15] I. Unk, I. Hajdú, K. Fátyol et al., "Human SHPRH is a ubiquitin ligase for Mms2-Ubc13-dependent polyubiquitylation of proliferating cell nuclear antigen," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 48, pp. 18107–18112, 2006.

[16] N. B. Watson, S. Mukhopadhyay, and W. G. McGregor, "Translesion DNA replication proteins as molecular targets for cancer prevention," *Cancer Letters*, vol. 241, no. 1, pp. 13–22, 2006.

[17] F. W. Larimer, J. R. Perry, and A. A. Hardigree, "The REV1 gene of *Saccharomyces cerevisiae*: isolation, sequence, and functional analysis," *Journal of Bacteriology*, vol. 171, no. 1, pp. 230–237, 1989.

[18] J. R. Nelson, C. W. Lawrence, and D. C. Hinkle, "Deoxycytidyl transferase activity of yeast REV1 protein," *Nature*, vol. 382, no. 6593, pp. 729–731, 1996.

[19] M. Diaz, J. Velez, M. Singh, J. Cerny, and M. F. Flajnik, "Mutational pattern of the nurse shark antigen receptor gene (NAR) is similar to that of mammalian Ig genes and to spontaneous mutations in evolution: the translesion synthesis model of somatic hypermutation," *International Immunology*, vol. 11, no. 5, pp. 825–833, 1999.

[20] P. E. M. Gibbs, X.-D. Wang, Z. Li et al., "The function of the human homolog of *Saccharomyces cerevisiae* REV1 is required for mutagenesis induced by UV light," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 8, pp. 4186–4191, 2000.

[21] D. R. Clark, W. Zacharias, L. Panaitescu, and W. G. McGregor, "Ribozyme-mediated REV1 inhibition reduces the frequency of UV-induced mutations in the human HPRT gene," *Nucleic Acids Research*, vol. 31, no. 17, pp. 4981–4988, 2003.

[22] S. Mukhopadyay, D. R. Clark, N. B. Watson, W. Zacharias, and W. G. McGregor, "REV1 accumulates in DNA damage-induced nuclear foci in human cells and is implicated in mutagenesis by benzo[a]pyrenediolepoxide," *Nucleic Acids Research*, vol. 32, no. 19, pp. 5820–5826, 2004.

[23] C. W. Lawrence, P. E. M. Gibbs, R. S. Murante et al., "Roles of DNA polymerase ζ and Rev1 protein in eukaryotic mutagenesis and translesion replication," in *Proceedings of the Cold Spring Harbor Symposia on Quantitative Biology*, vol. 65, pp. 61–69, 2000.

[24] A.-L. Ross, L. J. Simpson, and J. E. Sale, "Vertebrate DNA damage tolerance requires the C-terminus but not BRCT or transferase domains of REV1," *Nucleic Acids Research*, vol. 33, no. 4, pp. 1280–1289, 2005.

[25] J. G. Jansen, A. Tsaalbi-Shtylik, P. Langerak et al., "The BRCT domain of mammalian Rev1 is involved in regulating DNA translesion synthesis," *Nucleic Acids Research*, vol. 33, no. 1, pp. 356–365, 2005.

[26] A. Tsaalbi-Shtylik, J. W. A. Verspuy, J. G. Jansen et al., "Error-prone translesion replication of damaged DNA suppresses skin carcinogenesis by controlling inflammatory hyperplasia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 51, pp. 21836–21841, 2009.

[27] B. Zhao, J. Wang, N. E. Geacintov, and Z. Wang, "Polη, Polζ and Rev1 together are required for G to T transversion mutations induced by the (+)- and (-)-*trans-anti-BPDE-N^2*-dG DNA adducts in yeast cells," *Nucleic Acids Research*, vol. 34, no. 2, pp. 417–425, 2006.

[28] C. A. Dumstorf, S. Mukhopadhyay, E. Krishnan, B. Haribabu, and W. G. McGregor, "REV1 is implicated in the development of carcinogen-induced lung cancer," *Molecular Cancer Research*, vol. 7, no. 2, pp. 247–254, 2009.

[29] T. Sakiyama, T. Kohno, S. Mimaki et al., "Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk," *International Journal of Cancer*, vol. 114, no. 5, pp. 730–737, 2005.

[30] R. P. Young, R. J. Hopkins, B. A. Hay et al., "A gene-based risk score for lung cancer susceptibility in smokers and ex-smokers," *Postgraduate Medical Journal*, vol. 85, no. 1008, pp. 515–524, 2009.

[31] X. He, F. Ye, J. Zhang, Q. Cheng, J. Shen, and H. Chen, "REV1 genetic variants associated with the risk of cervical carcinoma," *European Journal of Epidemiology*, vol. 23, no. 6, pp. 403–409, 2008.

[32] K. H. Kraemer and H. Slor, "Xeroderma pigmentosum," *Clinics in Dermatology*, vol. 3, no. 1, pp. 33–69, 1985.

[33] J. E. Cleaver, "Xeroderma pigmentosumml: variants with normal DNA repair and normal sensitivity to ultraviolet light," *Journal of Investigative Dermatology*, vol. 58, no. 3, pp. 124–128, 1972.

[34] B. S. Tung, W. G. McGregor, Y.-C. Wang, V. M. Maher, and J. J. McCormick, "Comparison of the rate of excision of major UV photoproducts in the strands of the human HPRT gene of normal and xeroderma pigmentosum variant cells," *Mutation Research*, vol. 362, no. 1, pp. 65–74, 1996.

[35] A. R. Lehmann, S. Kirk Bell, and C. F. Arlett, "Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV irradiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 72, no. 1, pp. 219–223, 1975.

[36] J. C. Boyer, W. K. Kaufmann, B. P. Brylawski, and M. Cordeiro-Stone, "Defective postreplication repair in xeroderma pigmentosum variant fibroblasts," *Cancer Research*, vol. 50, no. 9, pp. 2593–2598, 1990.

[37] V. M. Maher, L. M. Ouellette, R. D. Curren, and J. J. Mccormick, "Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal human cells," *Nature*, vol. 261, no. 5561, pp. 593–595, 1976.

[38] C. Masutani, R. Kusumoto, A. Yamada et al., "The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η," *Nature*, vol. 399, no. 6737, pp. 700–704, 1999.

[39] R. E. Johnson, C. M. Kondratick, S. Prakash, and L. Prakash, "hRAD30 mutations in the variant form of xeroderma pigmentosum," *Science*, vol. 285, no. 5425, pp. 263–265, 1999.

[40] C. Masutani, M. Araki, A. Yamada et al., "Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity," *EMBO Journal*, vol. 18, no. 12, pp. 3491–3501, 1999.

[41] K. Watanabe, S. Tateishi, M. Kawasuji, T. Tsurimoto, H. Inoue, and M. Yamaizumi, "Rad18 guides polη to replication stalling sites through physical interaction and PCNA monoubiquitination," *EMBO Journal*, vol. 23, no. 19, pp. 3886–3896, 2004.

[42] C. Guo, J. N. Kosarek-Stancel, T.-S. Tang, and E. C. Friedberg, "Y-family DNA polymerases in mammalian cells," *Cellular and Molecular Life Sciences*, vol. 66, no. 14, pp. 2363–2381, 2009.

[43] L. S. Waters, B. K. Minesinger, M. E. Wiltrout, S. D'Souza, R. V. Woodruff, and G. C. Walker, "Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance," *Microbiology and Molecular Biology Reviews*, vol. 73, no. 1, pp. 134–154, 2009.

[44] Q. Lin, A. B. Clark, S. D. McCulloch et al., "Increased susceptibility to UV-induced skin carcinogenesis in polymerase η-deficient mice," *Cancer Research*, vol. 66, no. 1, pp. 87–94, 2006.

[45] K. Kuwamoto, H. Miyauchi-Hashimoto, T. Isei, and T. Horio, "Xeroderma pigmentosum variant associated with multiple cancers," *Photodermatology Photoimmunology and Photomedicine*, vol. 15, no. 3-4, pp. 127–132, 1999.

[46] K. H. Kraemer, M. M. Lee, and J. Scotto, "DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum," *Carcinogenesis*, vol. 5, no. 4, pp. 511–514, 1984.

[47] A. M. Flanagan, G. Rafferty, A. O'Neill et al., "The human POLH gene is not mutated, and is expressed in a cohort of patients with basal or squamous cell carcinoma of the skin.," *International Journal of Molecular Medicine*, vol. 19, no. 4, pp. 589–596, 2007.

[48] J. P. McDonald, V. Rapić-Otrin, J. A. Epstein et al., "Novel human and mouse homologs of *Saccharomyces cerevisiae* DNA polymerase," *Genomics*, vol. 60, no. 1, pp. 20–30, 1999.

[49] D. T. Nair, R. E. Johnson, S. Prakash, L. Prakash, and A. K. Aggarwal, "Replication by human DNA polymerase-ι occurs by Hoogsteen base-pairing," *Nature*, vol. 430, no. 6997, pp. 377–380, 2004.

[50] J. Wang, "DNA polymerases: hoogsteen base-pairing in DNA replication?" *Nature*, vol. 437, no. 7057, pp. E6–E7, 2005.

[51] G.-H. Lee, H. Nishimori, Y. Sasaki, H. Matsushita, T. Kitagawa, and T. Tokino, "Analysis of lung tumorigenesis in chimeric mice indicates the Pulmonary adenoma resistance 2 (Par2) locus to operate in the tumor-initiation stage in a cell-autonomous manner: detection of polymorphisms in the Poli gene as a candidate for Par2," *Oncogene*, vol. 22, no. 15, pp. 2374–2382, 2003.

[52] M. Wang, T. R. Devereux, H. G. Vikis et al., "Pol ι is a candidate for the mouse pulmonary adenoma resistance 2 locus, a major modifier of chemically induced lung neoplasia," *Cancer Research*, vol. 64, no. 6, pp. 1924–1931, 2004.

[53] G.-H. Lee and H. Matsushita, "Genetic linkage between Polι deficiency and increased susceptibility to lung tumors in mice," *Cancer Science*, vol. 96, no. 5, pp. 256–259, 2005.

[54] M. Obata, H. Nishimori, K. Ogawa, and G.-H. Lee, "Identification of the Par2 (Pulmonary adenoma resistance) locus on mouse chromosome 18, a major genetic determinant for lung carcinogen resistance in BALB/cByJ mice," *Oncogene*, vol. 13, no. 8, pp. 1599–1604, 1996.

[55] K. Bebenek, A. Tissier, E. G. Frank et al., "5′-deoxyribose phosphate lyase activity of human DNA polymerase ι in vitro," *Science*, vol. 291, no. 5511, pp. 2156–2159, 2001.

[56] R. C. Fernando, J. Nair, A. Barbin, J. A. Miller, and H. Bartsch, "Detection of 1,N6-ethenodeoxyadenosine and 3,N4-ethenodeoxycytidine by immunoaffinity/32P-postlabelling in liver and lung DNA of mice treated with ethyl carbamate (urethane) or its metabolites," *Carcinogenesis*, vol. 17, no. 8, pp. 1711–1718, 1996.

[57] S. Choudhury, S. Adhikari, A. Cheema, and R. Roy, "Evidence of complete cellular repair of 1,N6-ethenoadenine, a mutagenic and potential damage for human cancer, revealed by a novel method," *Molecular and Cellular Biochemistry*, vol. 313, no. 1-2, pp. 19–28, 2008.

[58] J. P. McDonald, E. G. Frank, B. S. Plosky et al., "129-Derived strains of mice are deficient in DNA polymerase ι and have normal immunoglobulin hypermutation," *Journal of Experimental Medicine*, vol. 198, no. 4, pp. 635–643, 2003.

[59] Y. Wang, R. Woodgate, T. P. McManus, S. Mead, J. J. McCormick, and V. M. Maher, "Evidence that in xeroderma pigmentosum variant cells, which lack DNA polymerase η, DNA polymerase ι causes the very high frequency and unique spectrum of UV-induced mutations," *Cancer Research*, vol. 67, no. 7, pp. 3018–3026, 2007.

[60] C. A. Dumstorf, A. B. Clark, Q. Lin et al., "Participation of mouse DNA polymerase ι in strand-biased mutagenic bypass of UV photoproducts and suppression of skin cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 48, pp. 18083–18088, 2006.

[61] T. Ohkumo, Y. Kondo, M. Yokoi et al., "UV-B radiation induces epithelial tumors in mice lacking DNA polymerase η and mesenchymal tumors in mice deficient for DNA polymerase ι," *Molecular and Cellular Biology*, vol. 26, no. 20, pp. 7696–7706, 2006.

[62] D. L. Mitchell, J. Jen, and J. E. Cleaver, "Relative induction of cyclobutane dimers and cytosine photohydrates in DNA irradiated in vitro and in vivo with ultraviolet-C and ultraviolet-B light," *Photochemistry and Photobiology*, vol. 54, no. 5, pp. 741–746, 1991.

[63] E. W. Newcomb, L. E. Diamond, S. R. Sloan, M. Corominas, I. Guerrerro, and A. Pellicer, "Radiation and chemical activation of ras oncogenes in different mouse strains," *Environmental Health Perspectives*, vol. 81, pp. 33–37, 1989.

[64] T. B. Petta, S. Nakajima, A. Zlatanou et al., "Human DNA polymerase iota protects cells against oxidative stress," *EMBO Journal*, vol. 27, no. 21, pp. 2883–2895, 2008.

[65] V. Poltoratsky, J. K. Horton, R. Prasad, W. A. Beard, R. Woodgate, and S. H. Wilson, "Negligible impact of pol ι expression on the alkylation sensitivity of pol β-deficient mouse fibroblast cells," *DNA Repair*, vol. 7, no. 6, pp. 830–833, 2008.

[66] H. Wang, W. Wu, H. W. Wang et al., "Analysis of specialized DNA polymerases expression in human gliomas: association with prognostic significance," *Neuro-Oncology*, vol. 12, no. 7, pp. 679–686, 2010.

[67] S. Zienolddiny, D. Campa, H. Lind et al., "Polymorphisms of DNA repair genes and risk of non-small cell lung cancer," *Carcinogenesis*, vol. 27, no. 3, pp. 560–567, 2006.

[68] P. Vineis, M. Manuguerra, F. K. Kavvoura et al., "A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility," *Journal of the National Cancer Institute*, vol. 101, no. 1, pp. 24–36, 2009.

[69] M. Luedeke, C. M. Linnert, M. D. Hofer et al., "Predisposition for TMPRSS2-ERG fusion in prostate cancer by variants in DNA repair genes," *Cancer Epidemiology Biomarkers and Prevention*, vol. 18, no. 11, pp. 3030–3035, 2009.

[70] G. Petrovics, A. Liu, S. Shaheduzzaman et al., "Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome," *Oncogene*, vol. 24, no. 23, pp. 3847–3852, 2005.

[71] S. A. Tomlins, D. R. Rhodes, S. Perner et al., "Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer," *Science*, vol. 310, no. 5748, pp. 644–648, 2005.

[72] M. Bienko, C. M. Green, N. Crosetto et al., "Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis," *Science*, vol. 310, no. 5755, pp. 1821–1824, 2005.

[73] N. Suzuki, E. Ohashi, A. Kolbanovskiy et al., "Translesion synthesis by human DNA polymerase κ on a DNA template containing a single stereoisomer of dG-(+)- or dG-(-)-*anti*-N^2-BPDE (7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene)," *Biochemistry*, vol. 41, no. 19, pp. 6100–6106, 2002.

[74] O. Rechkoblit, Y. Zhang, D. Guo et al., "trans-lesion synthesis past bulky benzo[a]pyrene diol epoxide N^2-dG and N^6-dA lesions catalyzed by DNA bypass polymerases," *Journal of Biological Chemistry*, vol. 277, no. 34, pp. 30488–30494, 2002.

[75] Y. Zhang, X. Wu, D. Guo, O. Rechkoblit, and Z. Wang, "Activities of human DNA polymerase κ in response to the major benzo[a]pyrene DNA adduct: error-free lesion bypass and extension synthesis from opposite the lesion," *DNA Repair*, vol. 1, no. 7, pp. 559–569, 2002.

[76] N. Guo, D. V. Faller, and C. Vaziri, "Carcinogen-induced S-phase arrest is Chk1 mediated and caffeine sensitive," *Cell Growth and Differentiation*, vol. 13, no. 2, pp. 77–86, 2002.

[77] X. Bi, D. M. Slater, H. Ohmori, and C. Vaziri, "DNA polymerase kappa is specifically required for recovery from the benzo[a]pyrene-dihydrodiol epoxide (BPDE)-induced S-phase checkpoint," *The Journal of Biological Chemistry*, vol. 280, pp. 22343–22355, 2005.

[78] S. Avkin, M. Goldsmith, S. Velasco-Miguel, N. Geacintov, E. C. Friedberg, and Z. Livneh, "Quantitative analysis of translesion DNA synthesis across a benzo[a]pyrene-guanine adduct in

mammalian cells: the role of DNA polymerase," *Journal of Biological Chemistry*, vol. 279, no. 51, pp. 53298–53305, 2004.

[79] S. Shachar, O. Ziv, S. Avkin et al., "Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals," *EMBO Journal*, vol. 28, no. 4, pp. 383–393, 2009.

[80] T. Ogi and A. R. Lehmann, "The Y-family DNA polymerase κ (pol κ) functions in mammalian nucleotide-excision repair," *Nature Cell Biology*, vol. 8, no. 6, pp. 640–642, 2006.

[81] T. Ogi, S. Limsirichaikul, R. M. Overmeer et al., "Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells," *Molecular Cell*, vol. 37, no. 5, pp. 714–727, 2010.

[82] D. Schenten, V. L. Gerlach, C. Guo et al., "DNA polymerase K deficiency does not affect somatic hypermutation in mice," *European Journal of Immunology*, vol. 32, no. 11, pp. 3152–3160, 2002.

[83] T. Shimizu, Y. Shinkai, T. Ogi, H. Ohmori, and T. Azuma, "The absence of DNA polymerase κ does not affect somatic hypermutation of the mouse immunoglobulin heavy chain gene," *Immunology Letters*, vol. 86, no. 3, pp. 265–270, 2003.

[84] J. N. K. Stancel, L. D. McDaniel, S. Velasco, J. Richardson, C. Guo, and E. C. Friedberg, "Polk mutant mice have a spontaneous mutator phenotype," *DNA Repair*, vol. 8, no. 12, pp. 1355–1362, 2009.

[85] J. O-Wang, K. Kawamura, Y. Tada et al., "DNA polymerase κ, implicated in spontaneous and DNA damage-induced mutagenesis, is overexpressed in lung cancer," *Cancer Research*, vol. 61, no. 14, pp. 5366–5369, 2001.

[86] Y. Wang, M. Seimiya, K. Kawamura et al., "Elevated expression of DNA polymerase kappa in human lung cancer is associated with p53 inactivation: Negative regulation of POLK promoter activity by p53," *International Journal of Oncology*, vol. 25, no. 1, pp. 161–165, 2004.

[87] V. Skaug, D. Ryberg, E. H. Kure et al., "p53 Mutations in defined structural and functional domains are related to poor clinical outcome in non-small cell lung cancer patients," *Clinical Cancer Research*, vol. 6, no. 3, pp. 1031–1037, 2000.

[88] M. Diaz, N. B. Watson, G. Turkington, L. K. Verkoczy, N. R. Klinman, and W. G. McGregor, "Decreased frequency and highly aberrant spectrum of ultraviolet-induced mutations in the hprt gene of mouse fibroblasts expressing antisense RNA to DNA polymerase ζ," *Molecular Cancer Research*, vol. 1, no. 11, pp. 836–847, 2003.

[89] Y. Murakumo, T. Roth, H. Ishii et al., "A human REV7 homolog that interacts with the polymerase ζ catalytic subunit hREV3 and the spindle assembly checkpoint protein hMAD2," *Journal of Biological Chemistry*, vol. 275, no. 6, pp. 4391–4397, 2000.

[90] S. K. Quah, R. C. Von Borstel, and P. J. Hastings, "The origin of spontaneous mutation in *Saccharomyces cerevisiae*," *Genetics*, vol. 96, no. 4, pp. 819–839, 1980.

[91] P. P. H. Van Sloun, I. Varlet, E. Sonneveld et al., "Involvement of mouse Rev3 in tolerance of endogenous and exogenous DNA damage," *Molecular and Cellular Biology*, vol. 22, no. 7, pp. 2159–2169, 2002.

[92] J. P. Wittschieben, S. C. Reshmi, S. M. Gollin, and R. D. Wood, "Loss of DNA polymerase ζ causes chromosomal instability in mammalian cells," *Cancer Research*, vol. 66, no. 1, pp. 134–142, 2006.

[93] M. Bemark, A. A. Khamlichi, S. L. Davies, and M. S. Neuberger, "Disruption of mouse polymerase ζ (Rev3) leads to embryonic lethality and impairs blastocyst development in vitro," *Current Biology*, vol. 10, no. 19, pp. 1213–1216, 2000.

[94] J. Wittschieben, M. K. K. Shivji, E. Lalani et al., "Disruption of the developmentally regulated Rev31 gene causes embryonic lethality," *Current Biology*, vol. 10, no. 19, pp. 1217–1220, 2000.

[95] G. Esposito, I. Godin, U. Klein, M.-L. Yaspo, A. Cumano, and K. Rajewsky, "Disruption of the Rev3l-encoded catalytic subunit of polymerase ζ in mice results in early embryonic lethality," *Current Biology*, vol. 10, no. 19, pp. 1221–1224, 2000.

[96] J.-M. Brondello, M. J. Pillaire, C. Rodriguez et al., "Novel evidences for a tumor suppressor role of Rev3, the catalytic subunit of Pol ζ," *Oncogene*, vol. 27, no. 47, pp. 6093–6101, 2008.

[97] K. Kawamura, J. O-Wang, R. Bahar et al., "The error-prone DNA polymerase zeta catalytic subunit (Rev3) gene is ubiquitously expressed in normal and malignant human tissues," *International Journal of Oncology*, vol. 18, no. 1, pp. 97–103, 2001.