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Translesion DNA Synthesis in Cancer: Molecular Mechanisms and Therapeutic Opportunities

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Abstract

The genomic landscape of cancer is one marred by instability, but the mechanisms that underlie these alterations are multifaceted and remain a topic of intense research. Cellular responses to DNA damage and/or replication stress can affect genome stability in tumors and influence the response of patients to therapy. In addition to direct repair, DNA damage tolerance (DDT) is an element of genomic maintenance programs that contributes to the etiology of several types of cancer. DDT mechanisms primarily act to resolve replication stress, and this can influence the effectiveness of genotoxic drugs. Translesion DNA synthesis (TLS) is an important component of DDT that facilitates direct bypass of DNA adducts and other barriers to replication. The central role of TLS in the bypass of drug-induced DNA lesions, the promotion of tumor heterogeneity, and the involvement of these enzymes in the maintenance of the cancer stem cell niche presents an opportunity to leverage inhibition of TLS as a way of improving existing therapies. In the review that follows, we summarize mechanisms of DDT, misregulation of TLS in cancer, and discuss the potential for targeting these pathways as a means of improving cancer therapies.

Graphical Abstract

CHALLENGES TO DNA REPLICATION ARE EVER PRESENT

The role of DNA replication in maintaining the integrity of the genome through the act faithful duplication is a central tenet of biology. The licensing, initiation, progression, and

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termination of replication forks involves a complicated interplay between numerous proteins and is subject to impediments that arise from both endogenous and exogenous sources.^{1,2} Evolution has retained multiple layers of protection against the untimely loss of information contained within the hereditary material, but nucleic acids are inherently reactive biological macromolecules and the covalent modification of DNA and RNA is ubiquitous.³ Moreover, the coordination of factors required to copy the ~6 billion base pair diploid human genome in a relatively short time is a complicated undertaking. Thus, every cycle of replication is faced with obstacles to the timely and accurate replication of the genome, obstacles that include DNA adducts, nucleotide depletion, ribonucleotide incorporation, misregulated origin licensing/firing, rereplication, oncogene activation, and naturally occurring replication fork barriers (RFBs), such as factors associated with transcription, non-B-form DNA structures, and fragile sites. The presence of any one of these factors can potentially lead to “stress” at the replication fork. The replication stress response (RSR) functions to overcome these obstacles, and as such, it is a vital part of ensuring that the genome is copied accurately and completely.⁴

MULTIPLE PATHWAYS AID IN THE RESOLUTION OF REPLICATION STRESS

Replication stress can be resolved through the combined actions of several mechanisms, including DNA repair, fork reversal, repriming of replication downstream of the block, firing of dormant origins, template switching, or translesion synthesis (TLS).⁴ Defects in the resolution of replication stress are associated with several cancer-prone human diseases.⁵ Template switching and TLS are two branches of DNA damage tolerance (DDT) that promote fork rescue/restart through bypass without repair/removal of the block to replication. Template switching is a homologous recombination-mediated response that involves fork regression and the use of the newly synthesized strand on the sister DNA duplex as a template for the pol.⁶ Template switching is coordinated by Rad5 in yeast,⁷ and even though Rad5 homologues SHPRH and HLF in humans are known to be involved in damage tolerance,^{8,9} the exact mechanism behind template switching in higher eukaryotes remains unclear.¹⁰ TLS is a DDT mechanism that is conserved from prokaryotes through eukaryotes and involves the use of specialized DNA polymerases (pols) to promote the direct bypass of DNA adducts and unusual template structures.

Generally speaking, TLS pols are inherently more error-prone than replicative pols, but the increased risk of TLS-induced mutation is less detrimental to genomic integrity than fork collapse, double-strand break (DSB) formation, and subsequent activation of programmed cell death. Of the DNA pols encoded in the human genome, approximately half have the ability to bypass DNA adducts.¹¹ Four Y-family members, Rev1, pol η , pol ι , and pol κ , are well-studied TLS pols with a versatile array of bypass abilities and mechanisms of action.¹² The catalytic subunit of pol ζ (Rev3L) is a B-family member that lacks exonuclease activity and plays a crucial role in TLS, often in coordination with Rev1.^{13–15} Other important TLS capable pols include the A-family members pols θ and ν and the X-family members pols β and λ .¹¹ The recently discovered PrimPol also exhibits several intriguing molecular and cellular attributes related to TLS.¹⁶ The general properties and catalytic features of these and other DNA pols have been reviewed extensively elsewhere and will not be discussed in detail.^{1,12,17} Conceptually, the central feature of TLS, namely, a set of proteins and enzymes

capable of catalyzing DNA synthesis across a barrier to fork progression, would seem to be a relatively simple solution to impaired replication. In reality, the recruitment and coordination of TLS components to sites of replication stress is a complex undertaking that depends on the cell cycle, genomic context, and type of lesion.

ATR SIGNALING IN RESPONSE TO REPLICATION STRESS

DNA adducts, nucleotide depletion, and endogenous RFBs can all induce slowing or stalling of the fork and uncoupling of the replicative polymerase/helicase, which results in the formation of ssDNA (Figure 1). Indeed, the act of unwinding the doublehelix to ssDNA intermediates during replication leaves the genome vulnerable to insult.^{18–20} The formation of persistent replication protein A (RPA)-coated ssDNA serves as a signal to the cell that a fork has encountered some impediment to progress, which can lead to activation of the intra-S-phase checkpoint mainly through the actions of ATR kinase.²¹ This response is not limited to slowed/stalled forks, as the end resection step during repair of double-strand breaks (DSBs) generates ssDNA that also leads to recruitment of the ATR–ATRIP complex.²²

The activation of ATR in response to replication stress involves a number of factors. The interaction between RPA-ssDNA and ATR-interacting protein (ATRIP) helps recruit ATR to sites of fork slowing/stalling.^{23,24} Further interactions involving the Rad17–RFC complex and the ring-shaped Rad9–Rad1–Hus1 (9–1–1) complex are important for stimulation of ATR kinase activity through recruitment of DNA topoisomerase II binding protein I (TopBP1).^{24,25} Binding of the 9–1–1 complex may be impacted by synthesis of short primers on both leading and lagging strands through the proposed action of pols α , δ , and κ .^{26,27} The enrichment of 5′-ends and primertemplate junctions near sites of stalled replication has been proposed to help improve binding of the 9–1–1 complex. Once activated, ATR phosphorylates a number of proteins including RPA (Ser33) and Chk1 (Ser345).²¹ RPA is hyperphosphorylated by several kinases, including ATM and DNA-PK, and likely helps to recruit additional targets for kinase activity to stalled and/or collapsed forks.²⁸

Phosphorylation of Chk1 activates the kinase activity of this cell cycle regulator.²⁹ Recruitment of Chk1 to sites of stress depends, at least in part, on interactions with RPA and the MCM complex.^{30,31} Additionally, pol α has been shown to interact physically with Chk1, facilitating TopBP1/ATR-dependent phosphorylation of the effector kinase Chk1 on Ser345.³² In general terms, the activation of ATR promotes fork stabilization and signals through the Chk1 kinase to promote cell cycle arrest, suppress recombination, and promote resolution of the block to replication through several processes including firing dormant origins, repriming replication downstream of the block, reversing the fork, or TLS.

TLS CAN OCCUR THROUGH BOTH PCNA UBIQUITINATION-DEPENDENT AND -INDEPENDENT MECHANISMS

Given the multitude of potential blocks to replication, it is perhaps not surprising that TLS action at sites of impaired fork progression is multifaceted and involves the coordination of many proteins. The switch from a high-fidelity enzyme to a pol capable of performing lesion

bypass is a central point of regulation. Recruitment of TLS in eukaryotes can be broadly divided into events that depend on ubiquitination (Ub) of the sliding clamp, PCNA, and those that occur without PCNA ubiquitination (Figure 1).^{33,34} The partitioning between these two TLS mechanisms is not completely understood, as it is almost certainly organism-specific and very likely depends on the type of block to replication.

In higher eukaryotes, Rev1 seems to be important for TLS that occurs without PCNA ubiquitination. Rev1 is an unusual enzyme because it uses a protein-template directed mechanism of action to primarily function as a cytidyl transferase. The Y-family pols η , ι , and κ possess a Rev1-interacting region (RIR) that binds to a region in the C-terminal domain of Rev1.^{35–38} This interaction recruits and stabilizes Y-family pols at sites of replication stress.^{33,39} In avian cells, deletion of the C-terminus of Rev1 results in defective replication of damaged DNA and G-quadruplexes.^{33,40} Although the Rev1 interaction with other Y-family members is conserved from flies through humans, the scaffolding function of Rev1 does not appear to be as important in yeast or worms, as recruitment of *Saccharomyces cerevisiae* pol η occurs independent of Rev1 in these organisms.^{41,42} The Rev7 and pol D3 subunits of pol ζ also interact with the C-terminal domain of Rev1 to promote TLS and regulate homologous recombination repair^{43–46}. In contrast to the interaction between pol η and Rev1, the indispensable nature of Rev1 in facilitating the recruitment and subsequent TLS action of pol ζ is conserved from yeast to humans.⁴²

As noted above, post-translational modification (PTM) of the sliding clamp is another important means of recruiting TLS pols to sites of replication stress with ubiquitination acting as the major modification responsible for facilitating the pol switch. Indeed, ubiquitination of PCNA is essential for TLS across UV-induced DNA damage in yeast.^{47,48} All of the human Y-family pols possess one or more ubiquitin binding motifs (Figure 2).¹² The ubiquitination of PCNA (either mono- or poly-Ub) promotes TLS by adding an additional anchor point between TLS pols and PCNA.¹² This is thought to strengthen the already existing interaction between PCNA-interacting peptides (PIP) on the pol and the sliding clamp. A notable exception to this rule is Rev1, which does not possess a PIP but instead relies upon a BRCA1 C-terminus (BRCT) domain to interact with PCNA.⁴⁹ An additional distinction may be made between PCNA-Ub-dependent mechanisms that occur at the fork during S-phase and those that occur in G2 phase as PRR, where a ssDNA gap is formed when replication restarts downstream from the block.³³ Other mechanisms of TLS regulation (e.g., transcriptional regulators, protein–protein interactions, phosphorylation, and other PTMs) have been discussed thoroughly elsewhere and will not be discussed in detail here.^{1,50}

The pathway choice for TLS depends on the lesion, and examples of both PCNA-Ub-dependent and -independent modes of TLS have been documented. The role of PCNA-Ub in the recruitment and retention of pol η represents an interesting example. Pol η -catalyzed bypass of UV-induced cyclobutane pyrimidine dimers (CPDs) is, in a sense, the prototypical TLS reaction, as the enzyme displays robust and accurate bypass of CPDs in vitro and mutations in the *Rad30* gene encoding pol η cause the sunlight-induced skin cancer-prone disease xeroderma pigmentosum variant (XPV).^{51–56} Cells that lack pol η exhibit defects in replication and increased mutagenesis after UV irradiation.^{57–60} Biochemical experiments

have revealed that pol η bypasses CPDs with essentially no impact on the catalytic efficiency and fidelity.^{52,55,56} Despite the clear connection between clinical phenotype and biochemical activity, defining the molecular mechanism used to facilitate the so-called “pol switch” during TLS past CPDs has proven challenging.

In a series of landmark studies, Rad18-mediated ubiquitination of PCNA at Lys164 was shown to facilitate recruitment of pol η in response to UV irradiation.^{61,62} The model that emerged from these initial studies placed the interaction between PCNA-Ub and the ubiquitin-binding motifs (UBM/ UBZ) of Y-family members at the center of TLS regulation. The Ub/UBZ interaction was proposed to strengthen the existing interaction between the noncanonical PCNA-interacting peptide (PIP) motifs of Y-family members and the sliding clamp. The damage-dependent ubiquitination of PCNA provided an elegant solution to the question of how TLS pol activity could be partitioned at the fork. Additional studies revealed that a constant push and pull exists between ubiquitination of the sliding clamp by Rad6/Rad18 and deubiquitination by USP1 with activation of DDR/RSR shifting the balance toward the ubiquitinated state.^{63,64} Furthermore, the *Rad5* homologues HLTF and SHPRH determine whether PCNA is mono- or polyubiquitinated, which helps partition specific pols to different types of lesions.⁹

Further study led to a model in which the ubiquitination of PCNA was not essential for TLS by pol η .^{65–67} Experiments in avian DT40 cells reported that fork progress was not disrupted in either *rad18*-deficient or *pcna*K164R cells (i.e., PCNA-Ub does not occur if Lys164 is mutated) treated with either UV or 4-nitroquinoline (4-NQO), whereas deletion of the TLS pol interacting domain of Rev1 led to slowed fork progress in response to these exogenous sources of DNA damage, leading to the proposed “on-the-fly” TLS mechanism.³³ Thus, PCNA-Ub is not required for recruitment/retention of pol η to sites of replication stress, but it probably increases the likelihood that TLS action by pol η will be successful.

Additional examples of PCNA-Ub-independent TLS have emerged. For example, PCNA-Ub is dispensable for pol η -catalyzed A/T mutagenesis during somatic hypermutation (SHM).⁶⁷ Bypass of UV-induced (6–4) photoproducts primarily proceeds through a PCNA-Ub-independent pathway involving Rev1 and pol ζ .⁶⁸ PCNA-Ub-independent TLS was also reported for Rev1-mediated bypass of G-quadruplex structures in DT40 cells.^{40,69} Finally, another Y-family member, pol κ facilitates bypass of DNA alkylation damage in the absence of PCNA ubiquitination.^{70,71} However, pol κ -catalyzed bypass of bulky adducts inflicted by BPDE requires PCNA-Ub, possibly through a mechanism involving polyubiquitination.^{71,72} Although the regulatory elements discussed in the previous sections help govern the partitioning between different pols at the fork, the success of TLS depends upon the structural and functional properties inherent to each enzyme for a given type of lesion.

TRANSLESION DNA SYNTHESIS USES SPECIALIZED ENZYMES TO RESOLVE DNA REPLICATION STRESS

Eukaryotes possess a versatile repertoire of DNA pols with diverse TLS capabilities. Although B-family pols do possess some TLS properties against abasic sites and small adducts, these replicative pols are often strongly inhibited by DNA adducts, and even if they

insert a base opposite the lesion, they either cannot readily extend past the lesion or they generate mis-pairs, which leads to a futile cycle of insertion and excision when the mis-pair is shuttled into the exonuclease “proofreading” active site.⁷³ Evolution has retained cellular responses and specialized replication components, including TLS pols, that assist in the bypass of adducts and other blocks to replicative pols.

TLS pols, such as the Y-family members, possess domain architectures that produce a solvent-exposed active site that is tolerant of distorted and/or damaged template DNA (Figure 2). In this regard, TLS pols provide two key advantages over replicative enzymes: first, they possess geometrically tolerant catalytic sites, and second, they do not proofread mis-pairs with exonuclease activity. They also tend to be much less processive than high-fidelity enzymes, which naturally limits the amount of DNA synthesis TLS pols will catalyze once they are granted access to the fork. TLS pols are able to bypass a wide variety of DNA adducts, but this comes at the expense of replication fidelity as the mis-insertion frequency of TLS enzymes can be orders of magnitude higher than replicative pols.⁷⁴ Indeed, the combined actions of pol ζ and Rev1 are responsible for the majority of DNA damage-induced single base mutations in mammalian cells.^{14,75} With that said, there are some instances where TLS is largely accurate, and both fork collapse and mutation may be avoided.

As mentioned before, a classic example of accurate and efficient TLS is pol η -catalyzed bypass of UV-induced CPDs. Another example of relatively accurate bypass of an offending lesion is pol κ -catalyzed bypass of bulky adducts, such as those induced by exposure to benzo[*a*]pyrene (B[*a*]P)-dG adducts. Multiple lines of evidence point to pol κ as a means of protecting mammalian cells from bulky DNA adducts, as pol κ -deficient cells exposed to BPDE exhibit elevated mutation frequencies and decreased survival.^{71,72,76} Pol κ expression is regulated by the aryl hydrocarbon receptor (AhR), which alters transcriptional profiles in response to polycyclic aromatic hydrocarbons.⁷⁷ Bioactivation of compounds, such as B[*a*]P, by cytochrome P450s upregulated in response to AhR activation can produce DNA adducts that, at least in some instances, are bypassed by pol κ . Additionally, the loss of pol κ results in a shorter lifespan and a mutator phenotype in *POLK*^{-/-} mice, again indicative of a protective role for TLS in maintaining genomic integrity.⁷⁸ Other examples include relatively accurate pol η -catalyzed bypass of 7,8-dihydro-8-oxo-deoxyguanosine (8-oxo-dG) and pol ν -catalyzed bypass of 5*S*-thymine glycol lesions. Several reports have also noted that TLS pols seem to exhibit greater accuracy against non-B-form structures than replicative pols,^{79–83} and loss of TLS pol action can lead to decreased replication efficiency at G-quadruplex sites.^{84,85}

The protective effects of TLS pols can be offset by the role of these enzymes in mutagenesis, and misregulated expression and/or aberrant recruitment of TLS pols to the fork can lead to increased genomic instability. For example, although the loss of pol κ is associated with increased sensitivity and mutagenesis following exposure to certain DNA damaging agents, the overexpression of pol κ leads to replication fork defects, elevated levels of endogenous DNA damage, increased homologous recombination, and chromosomal aberrations.^{86–89} Likewise, pol η protects from sunlight-induced skin cancer, but higher levels of pol η

promote resistance to anticancer drugs.^{90–92} Thus, like many biological processes, there is a double-edged nature to TLS that necessitates precise control.

TLS POLS ARE MISREGULATED IN CANCER

Genomic instability, a hallmark of cancer, is accelerated by defects in DNA replication and repair. Precancerous lesions often exhibit constitutively active DNA damage response (DDR) and RSR markers.^{93,94} Given the central role of TLS in DNA adduct bypass and the resolution of replication stress, it is not surprising that TLS pols and DDT components have been identified as factors in the etiology of multiple types of cancer (Table 1).

In 2005, Albertella et al. noted that several TLS pols (both X- and Y-family members) were aberrantly expressed in a variety of tumor types relative to normal tissue.⁹⁵ The DNA repair pols β and λ were overexpressed in ~30% of the tumor specimens.⁹⁵ Additional work found that Pol β expression correlates with resistance to several DNA damaging agents, including alkylating agents, cisplatin, and hydrogen peroxide.^{96,97} Several pol β mutants or splice variants have been identified that promote genomic instability in tumors (i.e., telomeric fusions, mutator phenotype).⁹⁸ Some of the pol β mutants appear to inhibit normal base excision repair. A positive correlation was observed between expression of pol λ in normal tissue and the smoking status of patients, whereas downregulation of pol λ was observed in smokers with advanced stage lung cancer.⁹⁹

The A-family member pol θ , which is involved in TLS and DSB repair following exposure to ionizing radiation, has been implicated as a prognostic indicator of poor outcomes for breast cancer patients.¹⁰⁰ The expression of pol θ is higher in multiple tumor types, including breast, colorectal, and nonsmall cell lung cancers.^{100–102} Like pol κ , ectopic expression of pol θ slows fork progression and induces chromosomal damage.¹⁰⁰ Attempts to identify small-molecule inhibitors of pol θ are ongoing and could prove to be valuable tools for the treatment of breast cancer.

POL κ IS OVEREXPRESSED AND CONTRIBUTES TO GENOMIC INSTABILITY AND CHEMORESISTANCE IN GLIOBLASTOMAS

Relative expression of the Y-family member pol κ has varied between tumor types. For example, the expression of pol κ was reduced in stomach and colorectal cancer.^{95,103} Conversely, pol κ expression was found to be higher in brain and lung cancer specimens.^{104–106} The clinical importance of pol κ in cancer is most firmly established for glioblastomas, where the aberrant activation of kynurenine (KYN) signaling through overexpression of tryptophan 2,3-dioxygenase (TDO) appears to promote upregulation of pol κ through the aryl hydrocarbon receptor (AhR) pathway.^{107,108} This leads to increased chromosomal damage and promotes resistance to Temozolomide (TMZ),^{87,109} a DNA alkylating agent often used in the treatment of glioblastoma. Reducing pol κ expression levels through either RNAi, inhibition of TDO activity or by blocking AhR activation leads to a reduction in the level of endogenous chromosomal damage.⁸⁷ Pol κ was also found to attenuate the cytotoxic properties of other DNA alkylating and DNA crosslinking agents, such as mitomycin C (MMC), methyl nitrosourea (MNU), and methylmethanesulfonate

(MMS).^{9,70,110,111} As glioblastoma specimens exhibit constitutive activation of the RSR, it is likely that pol κ contributes to RSR activation and intrinsic resistance to treatment observed in glioblastomas through dysregulation of fork dynamics and represents a target for improving treatment of this deadly form of cancer.

POL η FACILITATES RESISTANCE TO PLATINUM-BASED DRUGS AND SUPPORTS MAINTENANCE OF THE OVARIAN CANCER STEM CELL (CSC) POPULATION

The link between TLS and cancer was originally identified through study of XPV and pol η .^{51,52} Subsequent work has identified multiple instances where pol η may contribute to cancer progression and/or treatment. Expression of pol η was reportedly reduced in colorectal, lung, and stomach cancers but elevated in approximately two-thirds of head and neck mucosal-derived squamous cell carcinoma (HNSCC) specimens.^{112,113} Low expression of pol η was strongly associated with high complete response rate in HNSCC patients treated with cisplatin.¹¹² Additional studies found that, although the relative expression of pol η in lung and gastric adenocarcinomas was not changed from normal tissues, there was a significant inverse correlation between pol η expression and the survival of patients treated with platinum drugs.^{114,115} The clinical implications for pol η in de novo chemoresistance are further bolstered by in vitro assays and crystal structures, illustrating the proclivity of the enzyme to bypass intrastrand cross-links generated by platinum-based drugs.^{90,91,116–118}

Given the importance of pol η in patient response to chemotherapy, the discovery that pol η not only facilitates cisplatin resistance in ovarian cancer cells but that the enzyme serves to maintain the stem-like population in these tumors is of great interest. Cancer cells are a diverse population of cells that are morphologically and functionally different from each other within a tumor population. Tumor heterogeneity can arise due to mutations acquired by cancer cells over time. Clonal selection of a subpopulation of self-renewing CSCs (also called tumor-initiating cells) is an especially important aspect of tumor evolution. The CSC hypothesis states that a subpopulation of self-sustaining cells has the ability to self-renew, and there is evidence to suggest that CSCs are involved in tumor relapse; however, the mechanisms the underlie CSC participation in chemoresistance and tumor relapse have remained unclear. A recent study found that ovarian CSCs were more resistant to cisplatin compared to the bulk population and that this resistance was due, in large part, to elevated hpol η expression in the CD44⁺/CD117⁺ CSC population.¹¹⁹ Additional experiments using a xenograft mouse model of ovarian cancer validated the idea that hpol η was required for cisplatin-induced enrichment of CSCs in vivo and that downregulation of hpol η sensitizes ovarian tumors to CDDP treatment.¹¹⁹ These findings are consistent with the idea that higher hpol η levels in CSCs result in reduced efficacy of anticancer drugs and are potentially involved in mechanisms of acquired chemoresistance and tumor recurrence through maintenance of tumor-initiating cells.

REV1 AND POL ζ PROMOTE ACQUIRED RESISTANCE TO DNA DAMAGING AGENTS AND INCREASE TUMOR HETEROGENEITY THROUGH MUTAGENIC TLS

Although pol η is undoubtedly important for the development of resistance to platinum drugs, Rev1 and pol ζ are also central to mechanisms of chemoresistance and mutagenesis in tumors treated with genotoxic agents. In the case of cisplatin resistance, Rad18-mediated ubiquitination of PCNA helps coordinate Rev1, pol η , and pol ζ bypass of intrastrand cross-links, and Rev1 and pol ζ function independent of PCNA-Ub to facilitate repair of interstrand cross-links induced by cisplatin.¹¹⁷ Furthermore, depletion of either Rev1 or pol ζ reduces the rate at which tumor cells acquire resistance to cisplatin.^{120–122} Similarly, stable knockdown of Rev3L sensitizes *Eu-myc* lymphoma-derived tumors in mice to treatment with cyclophosphamide (CTX).¹²³ Suppression of Rev1 action does not have a major impact on survival of B-cell lymphoma cells exposed to CTX in culture. However, knockdown of Rev1 does reduce CTX-induced mutagenesis ~2–3-fold in cultured lymphoma cells and delayed the acquisition of CTX resistance in vivo.¹²³ The suppression of acquired resistance led to a significantly increased survival time in mice with Rev1-deficient tumors treated with CTX.

On the basis of the aforementioned studies, a clear role has been established for Rev1 and pol ζ -mediated TLS in the acquisition of mutations that promote acquired chemoresistance and increase tumor heterogeneity. The mutator phenotype hypothesis posits that the high mutation frequency within the cancer cell population causes tumors to evolve. New mutations are acquired by cancer cells, and compared to their counterparts, these mutant cells have a growth advantage that often includes the ability to resist treatments. Targeting these enzymes for downregulation or inhibition could be an effective means of extending the usefulness of drugs such as cisplatin and CTX. In a proof-of-principle study, siRNA encapsulated by nanoparticles to target Rev1 and Rev3L, the catalytic subunit of pol ζ , was used in combination with cisplatin to treat prostate cancer cells.¹²⁴ The knockdown of both hRev1 and hRev3 resulted in decreased drug-induced mutagenesis and sensitized cells to cisplatin. Moreover, treatment of xenograft murine models of prostate cancer with siRNA encapsulated by nanoparticles and cisplatin pro-drug resulted in 100% survival and no tumor growth for the duration of the experiment.¹²⁴

SMALL-MOLECULE INHIBITORS OF TLS POLS

Two approaches have commonly been pursued to leverage abnormalities in the DNA damage response (DDR) and replication stress response (RSR) in tumor cells to improve the treatment of cancer: (1) inhibit DDR/RSR responses to sensitize tumors to genotoxins or (2) identify patients with deficiencies in DDR/RSR to exploit synthetic lethal relationships. Both of these strategies have seen varying degrees of success in certain patient populations.^{125–129} Despite these advancements, failure to respond and resistance to existing treatments underscores the need for new approaches to create more effective treatments for patients that possess active DNA damage tolerance and repair mechanisms. Because TLS pols can render genotoxic anticancer drugs less effective through direct bypass of the lesions incurred

following treatment, and they are involved in mutagenesis following exposure to DNA damaging agents, which can facilitate resistance to cancer treatments, these unique enzymes represent an attractive target for the development of new anticancer therapies.

DNA replication has proven to be a useful target for anticancer and antiviral drugs.¹³⁰ Inhibitors of nucleotide synthesis, such as methotrexate, hydroxyurea, and 5-fluorouracil, continue to be used to treat multiple types of cancer, as well as psoriasis, rheumatoid arthritis, and sickle cell disease. DNA pol inhibitors, such as cytosine arabinoside, acyclovir, ganciclovir, and gemcitabine, are effective antivirals and remain an integral part of many cancer therapies.¹³¹ Topoisomerase inhibitors, checkpoint kinase inhibitors, and PARP inhibitors also target tumor cells by impairing DNA replication and repair.¹³¹

DNA damaging agents are routinely used in the treatment of multiple types of cancer. Genotoxic drugs, such as CTX, bleomycin, doxorubicin, MMC, and platinum-derived compounds, induce cytotoxic and antiproliferative effects through inhibition of genomic maintenance programs.¹³¹ The nonspecific effects of these agents on the surrounding tissue can result in acute toxicity and secondary tumor formation. For example, damage to cardiac muscle is a major problem with long-term exposure to drugs like DOX and a matter of some concern in the design of targeted cancer therapies.¹³² The use of anthracyclines is an especially insidious part of treating childhood acute lymphoblastic leukemia, as patients often have impaired skeletal muscle function, increased risk for chronic disease, and early mortality. Any advance in our ability to target the proliferative capacity and/or damage tolerance components of tumor cells in a specific manner is a step toward reducing patient risk for detrimental side-effects that can either occur during treatment or many years afterward.

The bypass of chemotherapy-induced DNA damage is especially problematic because it can (1) allow tumor cell survival, thereby reducing the effectiveness of the treatment and (2) if mutagenic can promote increased tumor heterogeneity. With growing evidence to support roles for damage tolerance pathways in cancer progression and treatment, investigators have turned their attention toward the development of therapeutics targeting TLS pols and factors associated with DDT. The functional properties and molecular architecture of TLS pols distinguish them from their replicative counterparts and serve as a basis for pursuing studies aimed at the development of small-molecule inhibitors of these enzymes.¹³³ Targeting nonessential TLS pols that serve to facilitate resolution of replication stress and chemoresistance in tumors could be one route to reducing toxic side effects and improving outcomes for patients treated with genotoxic drugs.

Attempts to identify and develop inhibitors of TLS pols have met with some success in recent years. Compounds targeting pols β , η , ι , κ , and λ with varying degrees of potency and specificity have been reported.¹³⁴ The inhibitors include synthetic and natural compounds, fatty acid and vitamin K3 derivatives, as well as nucleoside analogues. A pioneering study in 2009 developed a fluorescence-based strand-displacement assay amenable to miniaturization and high-throughput screening.¹³⁵ Three compounds were tested for inhibition of pols β , η , and ι to validate the utility of the assay. Later, a library of ~16,000 compounds were screened for inhibition of pol κ .¹³⁶ From these, candesarten

cilexetil, an angiotensin II receptor antagonist (Figure 2), was shown to inhibit recombinant pol κ with a low micromolar IC_{50} and could enhance UV-induced cytotoxicity in XPV cells. Another compound identified in the study from Yamanaka et al. was MK-886, a compound originally developed as an inhibitor of leukotriene biosynthesis. It was discovered in a subsequent study that MK-886 exhibits some specificity toward pol ι compared to other Y-family members.¹³⁷

Following up on these studies, our group screened a targeted library of compound for inhibition of pol η .¹³⁸ We identified an indole thiobarbituric acid (ITBA) derivative with an IC_{50} value of $\sim 30 \mu M$ against pol η . Modification of the ITBA scaffold increased the potency against pol η activity ~ 2 -fold. The presence of a 5-chloro substitution and the addition of an N-naphthyl substituent led to the greatest improvement in the potency of ITBA against pol η . Kinetic analysis revealed a nonlinear relationship between inhibitor concentration and the Michaelis constant ($K_{M,dTTP}$). This led us to propose a partial competitive mechanism of inhibition for the ITBA inhibitors in which the inhibitor-bound ternary complex (ESI) could still form product but at a slower rate than the ES complex. The initial molecular docking analysis was consistent with the kinetic model. The highest scoring poses for both PNR-3-84 and candesartan cilexetil were located in a cleft between the little finger and finger domains of pol η where the template strand normally resides. Given the variety of base pairing orientations observed in crystal structures of Y-family members, it is not a great leap to propose a model where ITBA-mediated distortion of the interaction between the pol and the template strand produces a complex that is not optimal for catalysis but can still result in product formation. We have since observed potentiation of cisplatin and doxorubicin by ITBA in multiple tumor cell lines (M.K. Zafar and R.L. Eoff, unpublished results).

Several reports have identified inhibitors of X-family pols that exert synergistic effects with DNA damaging agents in tumor-derived cell lines. Two studies from the laboratory of Andreas Marx have developed pol λ inhibitors from the rhodamine chemical scaffold.^{139,140} One of the most potent compounds inhibited pol λ with ~ 10 -fold greater potency than that of pol β and was able to potentiate the effects of hydrogen peroxide and TMZ in Caco-2 colorectal cancer cells. Very recently, an irreversible inhibitor of pol β lyase activity was found to potentiate the cytotoxicity of MMS in HeLa cells through inhibition of abasic site repair.¹⁴¹

A number of natural compounds have been shown to inhibit TLS pols in vitro and in some cases elicit antitumor responses in cell culture or animal models. For example, 3-Omethylfunicone, a compound isolated from an Australian fungal strain, was shown to inhibit pol κ with good selectivity.¹⁴² Another set of studies reported fairly broad inhibition of DNA pols and antiproliferative properties associated with glycolipids isolated from spinach.¹⁴³ Other studies have identified kohamic acid (KA-A) and penicilliois as fairly nonspecific DNA pol inhibitors with IC_{50} values in the low micromolar range.^{144,145}

Small-molecules targeting DDT factors beyond the TLS pols have also been identified. The PCNA/PIP-box interaction is disrupted by T2AA, a derivative of the potent thyroid hormone 3,3',5-triiodothyronine (T3).¹⁴⁶ Notably, the T2AA analogue of T3 disrupted the PCNA/PIP

interaction with an IC_{50} of 1 μM but does not exhibit any detectable thyroid hormone activity. Subsequent modification of the T3 scaffold improved the inhibition of TLS and also sensitized U2OS cells to cisplatin.^{146,147} Other DDT targets include the ubiquitin ligase Rad6B and deubiquitinase USP1. Although inhibitors of Rad6catalyzed ubiquitination have been identified, there is currently no evidence testing the ability of these compounds to inhibit either PCNA ubiquitination or TLS action.¹⁴⁸ Potent inhibitors of the USP1/UAF1 deubiquitinase complex have been identified, and several compounds appear to increase PCNA-Ub formation and enhance the antiproliferative effects of cisplatin;^{149,150} however, none of these compounds have been shown to impact TLS activity. It remains unclear how increased PCNA-Ub would have a detrimental impact on TLS action at sites damaged by genotoxic drugs as this would be expected to stabilize TLS pols and facilitate lesion bypass.

SUMMARY AND PERSPECTIVE

The multifaceted nature of TLS has proven to be a fascinating and nuanced subject that plays important roles in genomic maintenance programs. From the development of antibiotic resistance in bacteria to the role of TLS pols in cancer, the tolerance of DNA damage through TLS is an integral part of biological processes that impact human health. TLS appears to participate in mechanisms of both intrinsic and acquired resistance in multiple cancer types and, as such, represents an important target in the development of new therapeutics. Chemoresistance remains a significant obstacle to the treatment of many cancers, especially those for which early diagnosis of the disease is difficult, such as ovarian and pancreatic cancer. The potential benefits of targeting specialized pols must be weighed against the possibility that inhibiting DNA repair or TLS could result in chromosomal aberrations that promote secondary malignancies or other complications. As we've discussed, several preclinical studies seem to support the feasibility of using TLS pol inhibitors as either sensitizers to chemotherapy or as inhibitors of chemo-induced mutagenesis, but the therapeutic window for targeting TLS could be limited by several factors, including disease stage and whether or not the patient has already undergone treatment with genotoxic drugs. Promising preclinical studies have led to clinical trials targeting ATR and Chk1 kinases.¹⁵¹ Further development of inhibitor specificity, pharmacokinetic parameters, and patient stratification appear to be necessary, as preliminary clinical studies with Chk1 inhibitors UCN-01 and AZD7762 are not promising. The ATR/Chk1 trials were designed to test the ability to sensitize tumors to chemotherapy through inhibition of RSR signaling and may serve as a guide for future plans involving the development of tractable compounds targeting TLS pols. Targeting DDT more directly through inhibition of TLS pols or protein-protein interactions may prove to be a better strategy for adjuvant treatments. Advancing compounds that target TLS pols and other DDT factors through preclinical animal studies should remain a priority.

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Biography

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ABBREVIATIONS

AhR	aryl hydrocarbon receptor
BER	base excision repair
B[a]P	benzo[<i>a</i>]pyrene
BPDE	benzo[<i>a</i>]pyrene diol epoxide
BRCT	BRCA C-terminus domain
CPDs	cyclobutane pyrimidine dimers
CSC	cancer stem cell
CT	C-terminal
CTX	cyclophosphamide
DDR	DNA damage response
DDT	DNA damage tolerance
DSB	double-strand break
GBM	glioblastoma multiforme
HNSCC	head and neck mucosal-derived squamous cell carcinoma
HR	homologous recombination
Kyn	kynurenine
NER	nucleotide excision repair
NLS	nuclear localization signal
NSCLC	non-small cell lung cancer

PCNA	proliferating cell nuclear antigen
PIP	PCNA-interacting peptide
RIR	Rev1-interacting region
RSR	replication stress response
TDO	tryptophan-2,3-dioxygenase
TLS	translesion DNA synthesis
TMZ	Temozolomide
UBM	ubiquitin binding motif
UBZ	ubiquitin binding domain

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Figure 1. Overview of eukaryotic TLS. Cartoon illustration depicting key regulatory nodes and actions of proteins involved in TLS.

Figure 2.

Domain architecture of Y-family pols presents an interesting target for small-molecule inhibitors. (A) Schematic illustration of the domain structure associated with Y-family pols and the multisubunit TLS enzyme pol ζ . The core domains (finger, blue; palm, red; thumb, green; and little finger, purple) are shown for Y-family members along with the N-terminal extensions (orange) associated with Rev1 and pol κ . BD, binding domain; BRCT, BRCA C-terminal domain; CT, C-terminal domain; NLS, nuclear localization signal; PIP, PCNA-interacting peptide; RIR, Rev1-interacting region; UBM, ubiquitin binding motif; and UBZ, ubiquitin binding domain. (B) The structure of human DNA polymerase eta (hpol η) is shown with the finger (blue), palm (red), thumb (green), and little finger (purple) domains highlighted. The structure is overlaid with a cartoon depicting the right-handed nature of the TLS pol domain orientation (PDB ID: 3MR6). (C) Representative small-molecule inhibitors of TLS pols and DDT components.

General Bypass Properties of Primary TLS Pols and Roles in Cancer

Table 1.

notable bypass properties		role in cancer
Rev I	functions with pol ζ in mutagenic replication ^{45,68} acts as a scaffold protein for the other Y-family pols ^{41,45,152} promotes bypass and repair of DNA damage induced by platinum drugs ^{122,123,153} facilitates bypass of G-quadruplex DNA ^{40,69,81} incorporates dCMP opposite dG and abasic sites ^{154,155}	promotes acquired resistance to CTX and platinum-induced DNA damage through increased mutagenesis ^{120,122–124} germline missense mutations associated with an increased risk for cervical cancer ¹⁵⁶
pol κ	relatively accurate and efficient at bypass of several bulky minor groove adducts, such as benzo[<i>a</i>]-pyrene-N2-dG adducts participates in G-quadruplex replication ^{79,85} aids in the copying of microsatellite repeats ⁸³ functions in NER ¹⁶³	overexpression in glioblastomas through aberrant activation of KYN signaling ^{87,109} prognostic indicator for decreased survival in glioblastoma patients ¹⁰⁴ decreased level of transcripts in human colorectal tumors ¹⁰³ increased expression in lung cancer ^{105,106} promotes resistance to DNA damaging agents
pol η	accurate and efficient bypass of UV-induced CPDs ⁵⁶ bypasses platinum-induced intrastrand cross-links ^{116,157} participates in G-quadruplex replication ^{80,85} A-T mutator in somatic hypermutation of immunoglobulin variable genes ^{158,159} participates in replication of common fragile sites ^{160–162} extends D-loops in HR ¹⁶³	loss of pol η (in XPV) predisposes for sunlight-induced skin cancer ^{51,52} elevated expression in ovarian cancer stem cells and HNSCC ^{112,119} expression predicts patient response to platinum drugs in NSCLC, metastatic gastric carcinoma, and HNSCC ^{112,114,115} pol η -deficient cells are sensitive to doxorubicin ¹⁶⁴
pol ι	specialized Hoogsteen base-pairing mechanism of action ¹⁶⁵ replicates template dT in a highly error-prone manner ¹⁶⁶	increased expression in glioblastomas ¹⁰⁴ increased expression in breast cancer cell lines but not in breast cancer patient samples ^{167,168}
pol ζ	TLS across many types of DNA lesions ^{14,68,154} functions as an extender in coordination with RevI to bypass many types of DNA adducts ¹⁴ major role in DNA damage-induced mutagenesis ^{121,169,170}	decreased expression in lung, stomach, and colorectal cancer ¹¹³ promotes intrinsic and acquired resistance to platinum drugs and CTX ^{123,124}
pol θ	DNA repair of damage induced by ionizing radiation ^{122,123} introduces random nucleotides during alternative NHEJ ¹⁷¹ participates in DNA break repair near G-quadruplex sites ¹⁷² somatic hypermutation of immunoglobulin variable genes ¹⁷³	overexpression in breast cancer, colorectal cancer, and NSCLC ^{102,168,174,175} prognostic indicator for poor outcomes in breast cancer ^{100,175} ectopic expression impairs fork progression and increases chromosomal damage ¹⁶⁸

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role in cancerelevated expression observed in gastric, ovarian, prostate, thyroid, and uterine cancers⁹⁵mutants or splice variants expressed in approximately half of all cancers¹⁷⁹expression correlates with sensitivity to a wide range of genotoxic agents^{116,180,181}elevated expression in prostate and uterine cancer⁹⁵expression correlates with amount of smoking⁹⁹downregulated in advanced stage lung cancer of smokers⁹⁹**notable bypass properties**pol β involved in BER and may participate in meiotic recombination and mitochondrial DNA repair^{176–178}pol λ involved in BER, NHEJ, and V(D)J recombination^{182–184}