

VIEWPOINTS

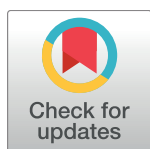
Inhibition of mutagenic translesion synthesis: A possible strategy for improving chemotherapy?

Kinrin Yamanaka¹, Nimrat Chatterjee¹, Michael T. Hemann^{1,2}, Graham C. Walker^{1*}

1 Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, **2** Koch Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America

These authors contributed equally to this work.

* gwalker@mit.edu



Overview

DNA damaging chemotherapy is the first line of treatment for certain cancers, but its long-term success is often marred by the eventual acquisition of chemoresistance. Other cancers cannot be treated because they are intrinsically resistant to such chemotherapy. These 2 types of resistance are coupled in the context of translesion synthesis (TLS), which is carried out by specialized TLS DNA polymerases that can replicate past DNA lesions but in a lower fidelity manner. First, TLS DNA polymerases permit the bypass of modified DNA bases during DNA synthesis, thereby allowing proliferation to continue in the presence of chemotherapy, an issue of particular relevance to intrinsic drug resistance. Second, mistakes introduced by TLS polymerases copying over DNA lesions introduced during the chemotherapy lead to mutations that contribute to acquired resistance. These dual functions of mutagenic TLS polymerases with respect to chemoresistance make these proteins very promising targets for adjuvant therapy. The major branch of mutagenic TLS requires REV1, a Y family DNA polymerase that recruits other TLS polymerases with its C-terminal domain (CTD) including POL ζ, which is also required. Recent evidence obtained using mouse models is summarized, which shows that interfering with REV1/POL ζ-dependent mutagenic TLS during DNA damaging chemotherapy can help overcome problems due to both intrinsic resistance and acquired resistance. Ways to develop drugs that block mutagenic TLS are also considered, including taking advantage of structural knowledge to target key protein-protein interfaces.

Introduction

While DNA damaging chemotherapy can be very effective and even curative in the treatment of certain cancers, intrinsic and acquired drug resistance underlies tumor progression and morbidity in many cancer patients. Intrinsic resistance defines a cell state that is inherently tolerant of drug action. This can include the activation of drug efflux pumps or detoxifying processes that effectively reduce intracellular drug concentration [1]. This can also include a change in the recognition or persistence of DNA damage, mediated by an enhanced DNA repair capability, a blunted DNA damage response, or the ability to proliferate in the presence of DNA damage. Conversely, acquired drug resistance represents a mutational or epigenetic process by which a chemosensitive cell develops 1 or more of the characteristics of an intrinsically resistant cancer cell. Thus, the mechanisms underlying intrinsic and acquired drug resistance are quite distinct. One describes a cell state, and the other describes the capability of

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reaching that cell state. Yet, these processes are very much coupled in the context of mutagenic translesion synthesis (TLS).

As discussed throughout this review, mutagenic TLS polymerases underlie 2 important phenotypes in response to genotoxic chemotherapy. First, they allow for the bypass of modified DNA bases during DNA synthesis, allowing proliferation to continue in the presence of chemotherapy. Second, the low fidelity replication performed by TLS polymerases results in the introduction of inappropriate, nonpairing bases across from modified nucleotides. The bypass function of TLS polymerases is particularly relevant to intrinsic drug resistance. Many tumors, including most pancreatic adenocarcinomas, nonsmall cell lung cancers, and aggressive brain tumors, as well as most metastatic malignancies, fail to significantly regress following chemotherapy [2]. In these tumors, TLS activity contributes to a drug resistant state by promoting the tolerance of DNA damage [3–6]. Conversely, the mutational role of TLS polymerases is central to process of acquired drug resistance. Tumor regression and relapse following chemotherapy is almost always accompanied by the development of drug resistant disease. This may not occur at initial relapse, but upon serial cycles of treatment patients generally succumb to tumors that have acquired intrinsically resistant disease. In fact, for certain cancers the overall prognosis is not dictated by the initial response of the tumor to chemotherapy. Rather, the response of the relapsed tumor to therapy is a significantly better determinant of overall survival. For instance, a high error-prone TLS activity translates into greater tumor adaptation to chemotherapy, while a low error-prone TLS activity leaves tumor in a treatment-naïve state. This latter state is amenable to continued long-term treatment of tumors that remain responsive to treatment with the initial therapy.

The dual functions of mutagenic TLS polymerases in intrinsic and acquired chemoresistance make these proteins very attractive potential targets for adjuvant therapy. When combined with front-line genotoxic therapy, these TLS inhibitors would be expected to sensitize tumors to chemotherapy while blocking drug-induced mutation. Consequently, while the generation of such inhibitors is complex, their route to the clinic is more apparent. TLS inhibitors could be applied in combination with the standard of care for many malignancies. By effectively increasing the effects of chemotherapy in target cells, these agents may also allow for a reduction in chemotherapy dose regimens. An added benefit of these agents may be a reduction in the rate of secondary chemotherapy-driven malignancies that occur in patients following successful treatment of the primary disease.

TLS polymerases bypass DNA damage

TLS polymerases are highly conserved, specialized DNA polymerases that can replicate past aberrant DNA lesions but in a lower fidelity manner—a trade-off that preserves genomic integrity in cells [7]. These incorrect nucleotides become fixed into mutations during the next round of DNA replication, contributing to overall fitness and evolution in single cell organisms but propelling tumorigenesis and disease in humans (Fig 1A). There are 10 known human TLS polymerases (REV1, POL η , POL ι , POL κ , POL ζ , POL μ , POL λ , POL β , POL ν , and POL θ), which are distributed in 4 families (Y, B, X, and A), and also Prim Pol, which additionally has primase activity. Although all TLS DNA polymerases are more error-prone than replicative DNA polymerases, some are capable of bypassing specific (cognate) lesions in a relatively error-free manner (Table 1). The extent of DNA synthesis errors during TLS depends on various factors, including the identities of the TLS polymerases employed, the presence or absence of cognate lesions, DNA sequence context, and thermodynamic favorability in the catalytic step [8–10]. The significance of the TLS process to human health is illustrated by xeroderma pigmentosum-variant patients, who are deficient in POL η and are

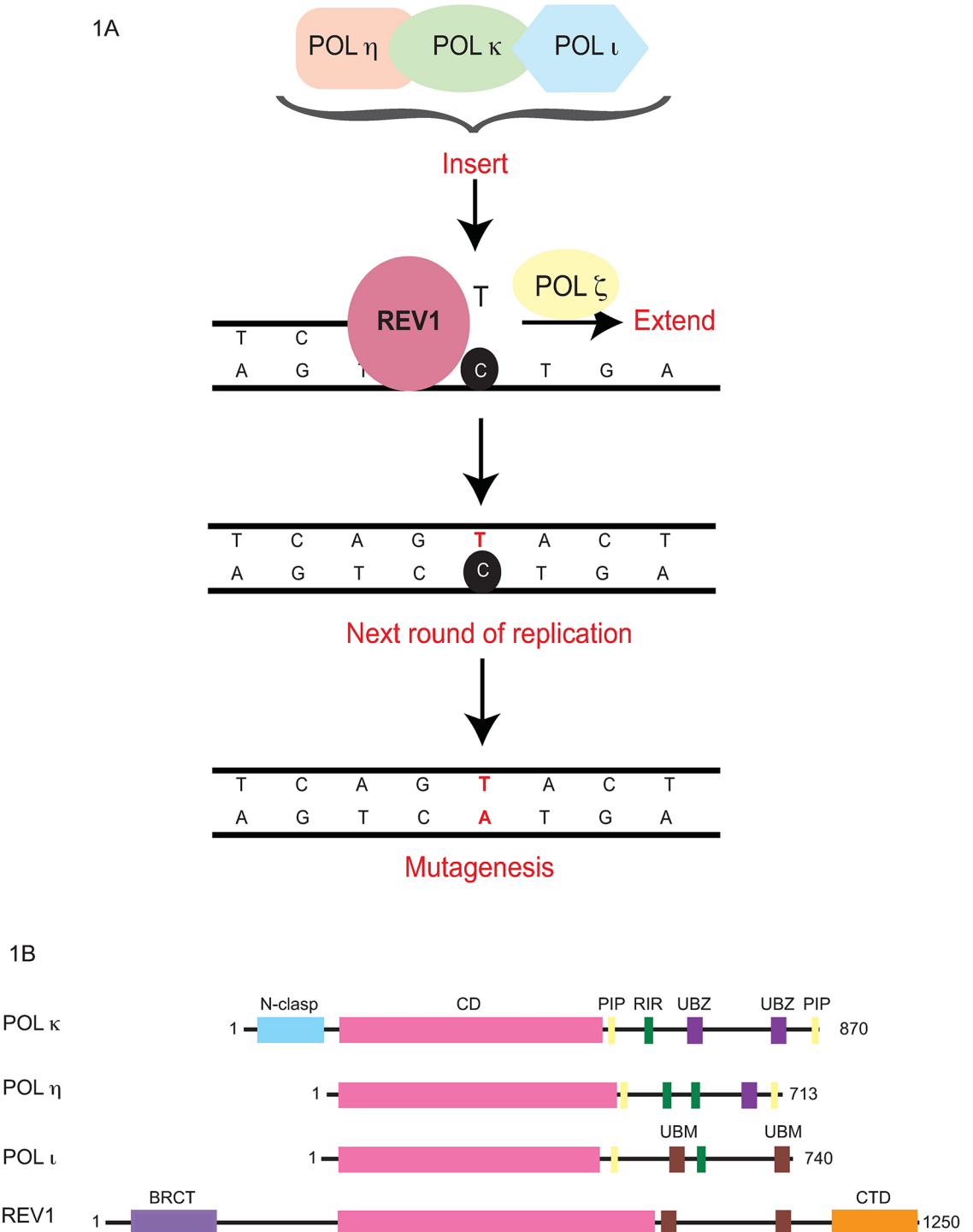


Fig 1. DNA damage bypass process. (A) Mechanism of the 2-step DNA damage bypass process. To bypass DNA damage, REV1 inserts deoxycytidine triphosphates across the damage or orchestrates the recruitment of the other polymerases, POL ι , POL κ , POL η , to replicate across the damage. Thereafter, POL ζ complex can help extend beyond the damage to enable re-initiation of undamaged DNA replication. If an incorrect nucleotide gets incorporated across the damage, this misincorporated nucleotide will lead to a mutation in the next round of replication. (B) A schematic representing the protein domains of the Y-family translesion synthesis (TLS) polymerases, REV1, POL ι , POL κ , POL η .

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Table 1. Summary of the characteristics, expression, the availability of mouse model, and association to cancers of B- and Y-family translesion synthesis polymerases.

Polymerase	Characteristics	Expression	Mice Model	Cancer Association
REV1 (<i>REV1</i>) Y-family	<ul style="list-style-type: none"> Exclusively inserts dCMPs opposite template Gs, abasic sites, and adducted G residues [13, 14] Acts as a scaffolding protein by interacting with both POL ζ and RIR containing POL η, POL κ and POL ι [15, 16] Generates G/C substitutions during Ig gene somatic hypermutation [17] Accumulates in DNA damaged induced foci [18–20] 	<ul style="list-style-type: none"> Protein expression is cytoplasmic in all tissues, with highest in adrenal gland, muscle, liver, etc. (http://www.proteinatlas.org/ENSG00000135945-REV1/tissue) RNA expressed in all tissues, with highest expression in brain tissues and reproductive organs (http://www.proteinatlas.org/ENSG00000135945-REV1/tissue and https://gtexportal.org/home/gene/REV1) 	<ul style="list-style-type: none"> <i>Rev1^{BRCT}</i> (ΔBRCT region; accelerated skin cancers, genotoxin-induced genome instability) [21, 22]. <i>Rev1^{AA}</i> (defective Rev1 catalytic domain; reduced somatic hypermutation) [23]. <i>Rev1^{KO}</i> (Rev1 deficient; near-infertile and unstable genome) [24] 	<ul style="list-style-type: none"> Several hepatocarcinomas and occasional lung cancers show high expression of REV1 [25] (http://www.proteinatlas.org/ENSG00000135945-REV1/cancer) Responsible for drug resistance in ovarian cancer cells [26] No known somatic mutations in cancers
POL η (<i>POLH</i>) Y-family	<ul style="list-style-type: none"> Bypasses T-T CPD and cisplatin-GG efficiently, but inefficiently across adducted residues, AP sites, 8-oxo-G [27–32] Accumulates at DNA damage foci [20, 33]. Generates A/T substitutions during somatic hypermutagenesis [34] 	<ul style="list-style-type: none"> Protein expression ubiquitous in nucleus and cytoplasm of all tissues, with high expression in thyroid, lung, pancreas, placenta, testis, etc. (http://www.proteinatlas.org/ENSG00000170734-POLH/tissue) RNA expressed in all tissues, with highest expression in tonsil, lymph nodes and testis (http://www.proteinatlas.org/ENSG00000170734-POLH/tissue and https://gtexportal.org/home/gene/POLH) 	<ul style="list-style-type: none"> <i>Polh^{KO}</i> (Pol η deficient; fertile, viable, but susceptible to skin cancers, mirrors XP-V phenotype, UV irradiated cells prone to chromatid breaks) [35–37] <i>Polh^{+/-}</i> (slightly susceptible to UV radiation-induced skin carcinogenesis) [35] 	<ul style="list-style-type: none"> Gene mutations causes XP-V [38] High expression in single basal cell carcinomas of the skin and some liver cancers (http://www.proteinatlas.org/ENSG00000170734-POLH/cancer) Enhanced expression in ovarian cancer stem cells [39] Elevated levels in head and neck tumor samples [40] 3 missense <i>POLH</i> mutations found amongst 201 melanoma patients [41]
POL κ (<i>POLK</i>) Y-family	<ul style="list-style-type: none"> Propensity to make –1 frameshift mutations, but efficiently bypasses thymine glycols and guanine adducts [42, 43] Propensity to extend mispaired primer-template termini [44] 	<ul style="list-style-type: none"> Protein expression data in normal tissues unknown RNA expressed in all tissues, with slightly high expression in thyroid, parathyroid, endometrium, and testis (http://www.proteinatlas.org/ENSG00000122008-POLK/tissue#gene_information & https://gtexportal.org/home/gene/POLK) 	<ul style="list-style-type: none"> <i>Polk^{KO}</i> (Pol κ deficient; fertile, cells are UV sensitive, spontaneous mutator phenotype in kidneys, liver and lungs, and the mice has shortened survival than <i>Polk^{+/-}</i> and <i>Polk^{+/+}</i> mice) [45, 46] 	<ul style="list-style-type: none"> Elevated expression in lung cancer [47, 48] Ectopic overexpression of POL κ induces aneuploidy and carcinogenesis in mice [49] Two non-coding <i>POLK</i> SNPs associated with lung cancer risk [50] Three somatic <i>POLK</i> mutations in 26 prostate patients [51]
POL ι (<i>POLI</i>) Y-family	<ul style="list-style-type: none"> Efficiently bypasses template dA; but does so inefficiently on the template dT [52, 53] Briefly accumulates in replication stress foci [54] Back-up polymerase in the absence of POL η. Inefficiently bypasses UV damage in the absence of POL η [11, 55] 	<ul style="list-style-type: none"> High protein expression in parathyroid, thyroid, reproductive organs and pituitary (http://www.proteinatlas.org/ENSG00000101751-POLI/tissue) High RNA expression in testis, thyroid and parathyroid gland (http://www.proteinatlas.org/ENSG00000101751-POLI/tissue and https://gtexportal.org/home/gene/POLI) 	<ul style="list-style-type: none"> <i>Poli^{KO}</i> (Pol ι deficient; mice susceptible to damage-induced lung tumors) [56]. <i>Poli^{KO}</i> mice cells not sensitive to DNA damaging agents [57] 	<ul style="list-style-type: none"> Elevated expression in breast cancer cells [58] Important candidate for lung neoplasia [59] Overexpressed in bladder cancer and in esophageal squamous cell carcinoma [60–62] <i>POLI</i> SNP (rs8305) correlated with significant high risk of both lung adenocarcinoma and squamous cell carcinoma [63] <i>POLI</i> SNP (rs3218786) significantly associated with TMPRSS2-ERG fusion-positive prostate tumors [64]

(Continued)

Table 1. (Continued)

Polymerase	Characteristics	Expression	Mice Model	Cancer Association
POL ζ_4 B-family (REV3 [REV3] polymerase, REV7 [REV7], POLD2 and POLD3 accessory subunits) [65]	<ul style="list-style-type: none"> • POL ζ_4 mediate inefficient TLS across CPDs, (6–4) photoproducts, adducted residues and AP sites, but an error free bypass of thymine glycols [53, 66, 67] • Serves as the key extender polymerase during TLS [68] 	<ul style="list-style-type: none"> • REV3 protein is expressed minimally in the cytoplasm of different tissue types. REV3L transcript is highly expressed in endometrin, smooth muscle, cerebellum and the uterine tissues (http://www.proteinatlas.org/ENSG00000009413-REV3L/tissue and https://gtexportal.org/home/gene/REV3) • High REV7 protein expression in bone marrow and lung tissues. And high REV7 RNA expression in testis, bone marrow, lymph nodes, tonsils, and appendix (http://www.proteinatlas.org/ENSG00000116670-MAD2L2/tissue and https://gtexportal.org/home/gene/MAD2L2) 	<ul style="list-style-type: none"> • <i>Rev3^{KO}</i> (Rev3 deficient; embryonically lethal and spontaneous and genotoxin induced genome instability) [69–71] • <i>Rev3^{Allox}</i> (conditional Rev3 deficiency; reduced cell proliferation, spontaneous genomic instability and mice develop spontaneously mic lymphoma and spontaneous skin tumors) [72–74] • <i>Rev7^{KO}</i> (Rev7 deficient; delayed growth, infertile, reduced cell proliferation, spontaneous genome instability) [75, 76] 	<ul style="list-style-type: none"> • REV7 depletion enhances cisplatin sensitivity in ovarian cancer cells [77] • Loss of REV7 sensitizes ovarian and breast cancer cells to PARP inhibition [78] • High expression in B-cell lymphoma [79] • Elevated expression in colon cancer [80]

AP, apurinic; CPD, cyclobutane pyrimidine dimers; dCMP, deoxycytidine monophosphate; TLS, translesion synthesis; XP-V, xeroderma pigmentosum-variant.

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therefore susceptible to UV radiation-induced cancers because the cognate UV-induced cyclobutane pyrimidine dimers are instead bypassed by alternate TLS polymerases (POL ι and POL κ) in a relatively error-prone manner [11, 12].

Distinct structural and biochemical features of the TLS polymerases enable them to replicate past the DNA damage. For example, in contrast to classical replicative polymerases, Y-family TLS polymerases possess a smaller thumb and finger domain that makes fewer contacts with DNA and also lack an 3'-5' exonuclease activity to proofread misincorporated nucleotides. Together, these structural attributes result in a larger and/or more permissive catalytic site than replicative polymerases that allows TLS polymerases to accommodate distorted and damaged nucleotides [81, 82]. In addition, other physical features such as the polymerase-associated domain of Y-family polymerases and the wrist and the N-clasp region of POL κ also contribute to polymerase architecture conducive to replication across DNA damage (Fig 1B) [83–87]. Furthermore, regulatory domains of TLS polymerases enable their proper localization and regulation [88]. These special structural features of TLS polymerases are fundamental to their roles in DNA damage bypass.

Besides the structural features of individual TLS polymerases, successful TLS also depends on interactions between these polymerases and other cellular proteins that target and choreograph their activity. REV1 functions as a principle scaffolding protein, which recruits other TLS polymerases to first insert a nucleotide opposite the DNA lesion and then eventually help extend the distorted primer-template terminus, in what is recognized as the two-step mechanism of TLS (Fig 2) [7, 8, 89]. For the insertion step, a particular interface of the REV1 CTD interacts with the REV1-interacting-region (RIR) of the inserter polymerases (POL η , POL ι , POL κ). Mutations that disrupt the RIR-interface in the Rev1 CTD prevent interaction with the inserter polymerase in yeast-2 hybrid (Y2H) screens [15, 16, 90, 91]. Insertion across from the damaged base can also be less frequently carried out by REV1 and POL ζ [8]. In the second step, an extender TLS enzyme, a role most frequently fulfilled by POL ζ (REV3/REV7/POLD2/POLD3) and in some cases by POL κ , replaces the inserter and extends the primer-template

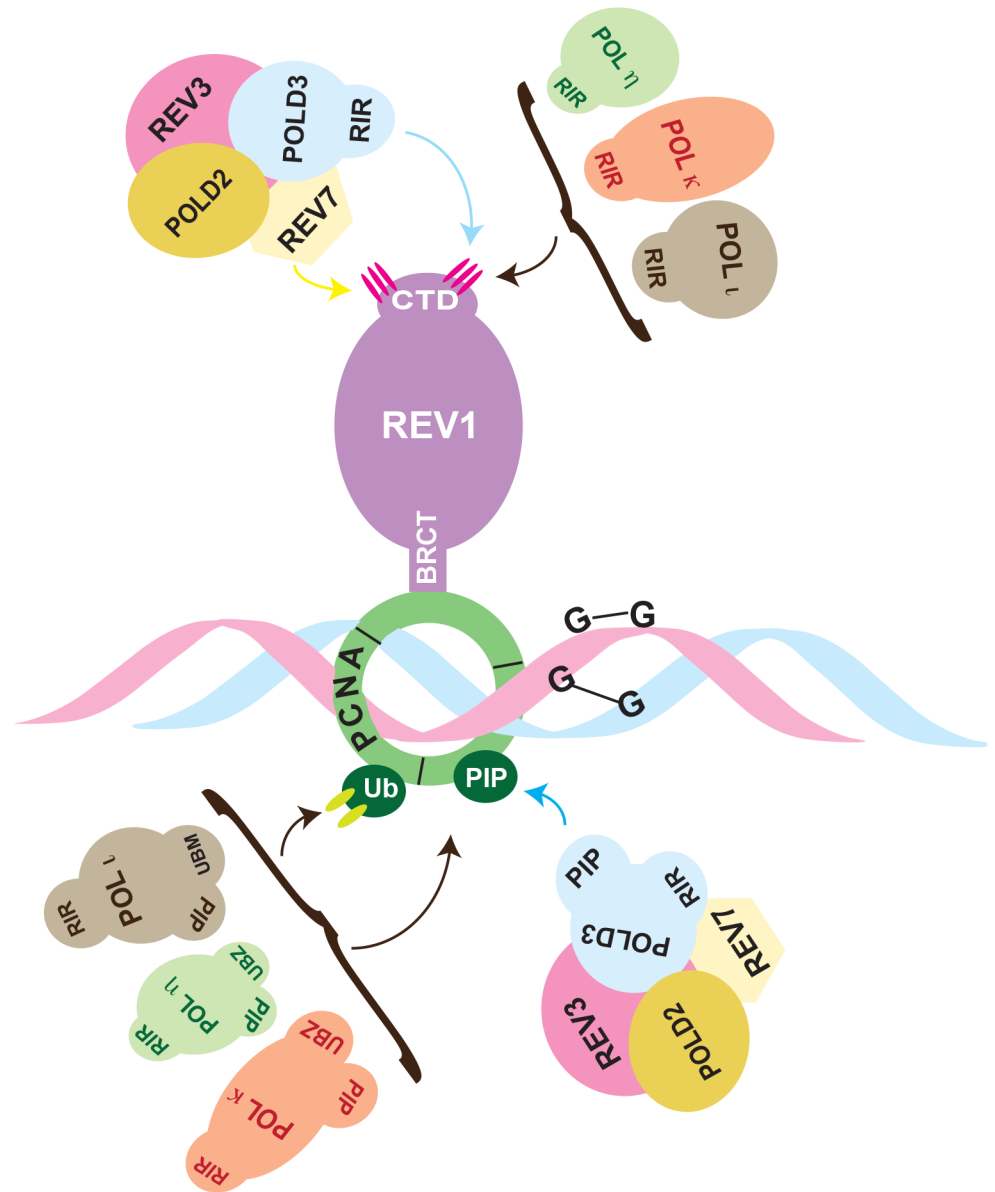


Fig 2. Protein-protein interactions between translesion synthesis (TLS) polymerases are important for the DNA damage bypass process. Two pathways are expected to facilitate TLS across DNA damage—the REV1 dependent and REV1 independent pathway. Majority of the DNA lesions are bypassed in a REV1 dependent fashion, which engages in protein-protein interactions with other TLS polymerases via its C-terminus. REV1 interacts with the REV1-interacting-region (RIR)-containing residues of POL ι , POL κ , POL η to enable insertion of nucleotides across the damage. And REV1 also interacts via key residues with REV7 of the POL ζ complex to facilitate extension beyond the insertion step. REV1 also binds to POLD3 subunit of the POL ζ complex to enable the key switch from the “insertion” to the “extension” step. In the REV1 independent pathway, the RIR-containing polymerases, POL ι , POL κ , POL η , by interacting with the proliferating cell nuclear antigen (PCNA) interacting protein (PIP) and ubiquitin-binding motif (UBM)/ ubiquitin-binding zinc finger (UBZ) domains of PCNA, can also enable TLS at the damaged site. Likewise, the POL ζ complex also interacts with the PIP box of PCNA to access the DNA and enable TLS.

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termini [90]. For the POL ζ -mediated extension step, a different interface in REV1 CTD—distinct from the interface for RIR recognition—makes contact with specific amino acids located on REV7. Mutating residues in the Rev7-interface of the Rev1 CTD inhibits Rev1-Rev7

interaction in Y2H studies and sensitizes chicken DT40 cells to cisplatin [15]. Apart from bypassing DNA damage at stalled replication forks, TLS polymerases also engage in filling single stranded (ss) DNA gaps left behind by replicative polymerases, via the less-well understood gap-filling mechanism [92, 93].

Interestingly, TLS polymerases are also required for other cellular functions. For example, during interstrand cross-link (ICL) repair in replicating cells, certain TLS polymerases—REV1, POL ι , POL κ and POL ν —are required for DNA synthesis over the ICL on the newly exposed leading strand [94–97]. Likewise, in nonreplicating cells, ICL repair depends on the Rev1-POL ζ TLS polymerases to fill the ssDNA-gaps [98]. In a similar fashion, both nucleotide excision repair (NER) and base excision repair (BER) pathways respectively can employ POL κ and POL η to fill the ssDNA gaps left behind after the excising step [99, 100]. Additionally, POL η was recently shown to drive microhomology-mediated break-induced replication (MMBIR) that causes complex genomic rearrangements in yeast and has an important role in homologous recombination (HR) in DT40 cells [101, 102]. Finally, REV1 was recently shown to be required for replication of G-quadruplex structures, thereby influencing epigenetic stability [103]. Independent of its role in TLS, REV7 promotes nonhomologous end joining (NHEJ) at double strand breaks and at telomeres by inhibiting CtIP-mediated end resection [104]. Additionally, REV7 plays a supporting role in cell cycle regulation by sequestering CDH1, which prevents premature activation of the anaphase-promoting complex, thereby inhibiting an exit from mitosis [105]. All these examples are suggestive of an overarching influence of TLS polymerases and their components on cellular physiology, in which they influence DNA damage tolerance, DNA repair, epigenetic stability, and replication across repetitive sequences.

Modulation of TLS polymerases alters tumor response to chemotherapy

A growing body of evidence now shows that suppression of TLS polymerases not only sensitizes tumor cells to drugs, but also reduces acquisition of drug-induced mutations implicated in tumor resistance. Thus, inhibition of TLS polymerases is a promising new approach to improving cancer therapy. Moreover, in some cancers, TLS polymerases are overexpressed (Table 1),

The impact TLS polymerases have on chemotherapy responses in different cancer subtypes has recently been investigated. In one study, the potential of Rev3 inhibition for the treatment of intrinsically chemoresistant cancers was investigated. A study utilizing the *Kras*^{G12D}; *p53*^{-/-} preclinical model of lung adenocarcinoma showed that, when the level of Rev3 was reduced, these otherwise resistant tumors were sensitized to cisplatin, increasing the overall survival of mice with Rev3-deficient tumors by 2-fold compared with control mice with Rev3-proficient tumors [106]. Reduction of Rev3 or Rev1 in these tumor cells also reduced cisplatin-induced mutagenesis in culture.

In a study that employed the *Eu-myc arf*^{-/-} mouse model of B-cell lymphoma, when mice were subjected to repeated cycles of tumor engraftment and cyclophosphamide treatment, relapsed tumors that appeared after the first round of chemotherapy continued to respond to cyclophosphamide if they were Rev1 deficient. This is in direct contrast to Rev1-proficient relapsed tumors, which exhibited varying degrees of acquired resistance to cyclophosphamide chemotherapy (Fig 3). Additionally, cyclophosphamide-induced mutagenesis of these lymphoma cells in culture was suppressed by Rev1 depletion. These studies showed that Rev1-dependent error-prone bypass of cyclophosphamide-induced DNA damage contributes to the mutagenesis and hence the tumor drug resistance. Thus this study provided the first in vivo evidence that TLS polymerases play a critical role in the development of acquired chemoresistance [107].

Chemotherapy-induced mutagenesis is a phenomenon proposed to cause secondary malignancies and tumor relapse. Hence, targeting REV1 and REV3 might not only increase killing

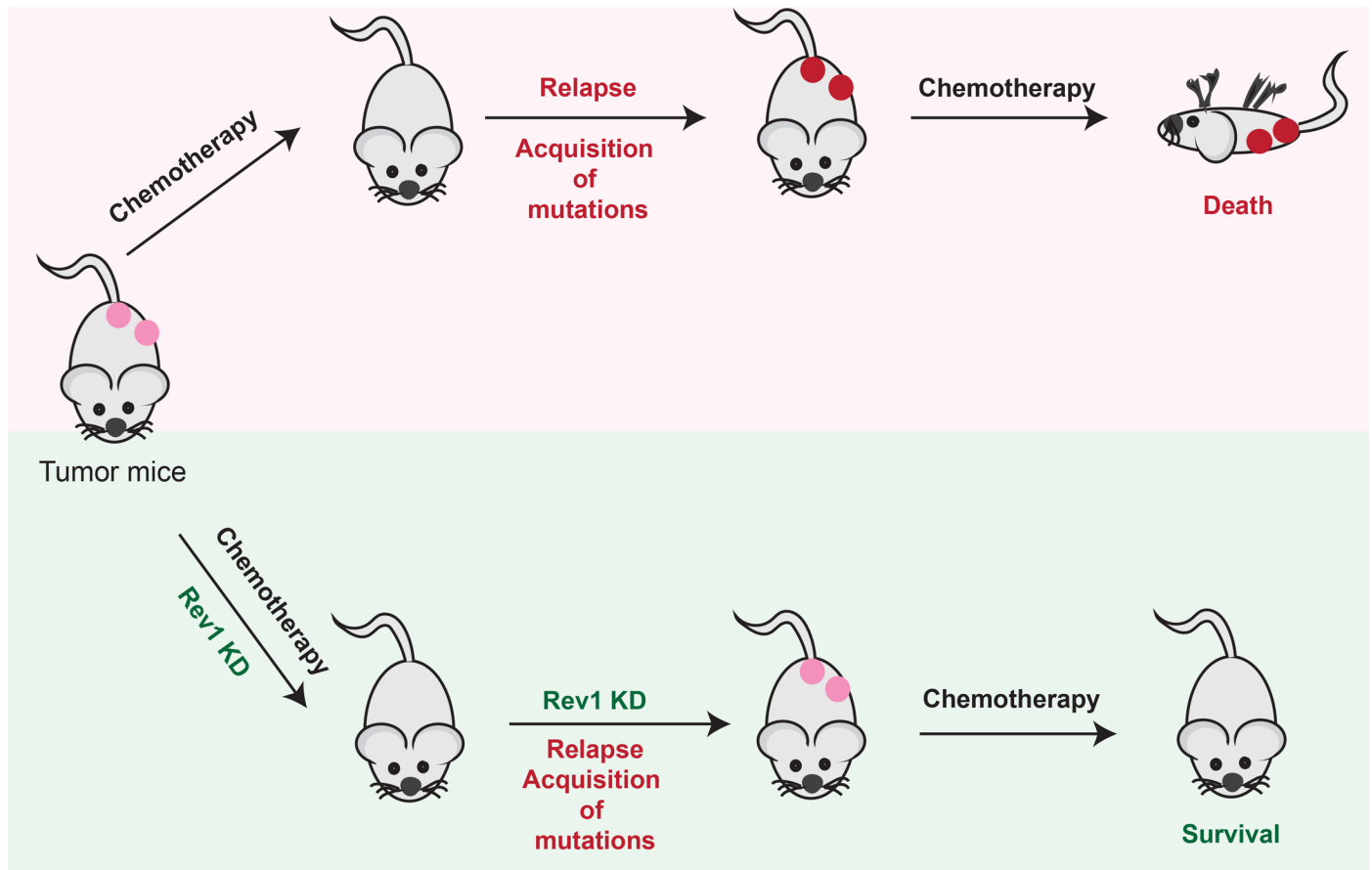


Fig 3. Reduction of Rev1 suppresses chemoresistance. In a tumor mouse model, administration of chemotherapy reduces tumor formation by killing the generally chemoresensitive tumor cells. However, many of the tumors that relapse are resistant to further killing from chemotherapeutic treatment, thereby reducing survival of the mice. In contrast, mice harboring relapsed tumors in which REV1 has been knocked down remain sensitive to chemotherapy, whereby their survival is prolonged.

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of cancer cells but could also potentially suppress secondary malignancies and tumor relapse. The same principal was explored when an innovative nanoparticle-mediated delivery system was used to target both REV1 and REV3 in combination with a cisplatin prodrug. A nearly complete inhibition of tumor growth and dramatically enhanced survival was observed in LnCaP prostate cancer mouse model [108]. In addition, REV7 depletion has been shown to sensitize ovarian cancer to cisplatin and reduce tumor volumes in nude mice [77]. These studies support the hypothesis that TLS inhibition can suppress at least some classes of intrinsic chemoresistance. Likewise depletion of REV3 in cervical cancer cells [109] or nonsmall cell lung cancer cells [110]; REV1, POL ζ , POL η in HeLa cells [111]; and POL η in ovarian cancer stem cells [39] all sensitize cells to cisplatin. It remains to be seen whether other cancer cell subtypes would similarly respond to knockdown of TLS polymerases and whether observations in cell studies could be recapitulated in mouse models.

Another approach to potentially enhance tumor cell killing via suppression of TLS polymerases is to discover synthetic lethal partners of TLS polymerases. For example, this classical approach is employed in killing BRCA2-deficient tumors by utilizing PARP1 inhibitors [112]. Although a compelling idea, TLS synthetic partners are largely unknown. However, a whole genome siRNA library screen in A549 lung cancer cells identified one gene RRM1—the large

subunit of ribonucleotide reductase that confers a synthetic lethal interaction with REV3 [113]. In another lung cancer cell line and in breast cancer cells, ataxia-telangiectasia and Rad3 related inhibition was found to synthetically enhance lethality in cisplatin-treated REV3-deficient cells [114]. In addition, Rev3-deficient DT40 cells exhibited synthetic lethality with RAD54 [115], suggesting a promising potential. Synthetic-lethal partners of TLS polymerases need to be explored in greater detail across other cancer subtypes.

Drug inhibitors to target TLS polymerases

Taken together, the studies discussed above suggest that small molecules that directly inhibit catalytic functions or disrupt key protein-protein interactions of TLS polymerases could be adjuvants that have the potential to significantly improve chemotherapy. For example, fluorescence-based assays conducted in high-throughput platforms were used to search for small molecule inhibitors that affect catalytic functions of TLS polymerases. Pamoic acid, aurintricarboxylic acid, and ellagic acid were found to inhibit POL ι and POL η [112], while candesartan cilexetil inhibited the enzymatic function of POL κ as well as enhanced UV-induced cytotoxicity in xeroderma pigmentosum-variant (XP-V) cells [116]. Likewise, 3-*O*-methylfunicone, a natural compound isolated from a marine fungal strain, selectively inhibited mammalian Y-family TLS polymerase activity (POL κ , POL ι , POL η) [117]. Further studies are required to identify compounds with improved specificity and potency.

Very recently small molecules inhibitors that target TLS DNA polymerase protein-protein interactions have been shown to be possible therapeutic candidates. For example, a small molecule inhibitor that binds to REV7 and inhibits its interaction with REV3 was shown to partially suppress ICL repair [118]. Whether the same drug could also suppress TLS is worth investigating. Similarly, detailed structural knowledge of other TLS interfaces, such as between REV1 and REV7 and between REV1 and RIR carrying proteins could be exploited in drug discovery and design.

Perspective and conclusion

Inhibiting TLS polymerases is a promising approach to improve chemotherapy as it could increase killing of cancer cells, while at the same time reducing the possibility of relapse and acquired drug resistance by reducing chemotherapy-induced mutagenesis. Even cancers known to be intrinsically drug resistant could potentially be sensitized by this approach. Additionally, TLS specific inhibition could also potentially target other repair and recombination pathways that involve TLS polymerases including NER, BER, MMBIR, HR, and NHEJ. However, several outstanding questions still need to be addressed, for example, improving understanding of the structural basis of key protein-protein interactions made by the TLS polymerases. Recently it was shown that the subunits of replicative polymerases cross talk with TLS Polymerases. For instance, the POLD3 subunit of the replicative DNA polymerase POL δ possess an RIR that interacts with the RIR-interface of REV1 CTD, while the POLD2 subunit of POL δ interacts with POL η [90]. These observations suggest that the TLS mechanism is even more complex than previously anticipated and that drug inhibitors for 1 TLS polymerase could potentially target multiple other TLS polymerases. An added complication is that TLS polymerases η , ι , and κ can also function independently of REV1 by interacting with proliferating cell nuclear antigen (PCNA) via the UBM/UBZ domain and the PCNA interacting protein (PIP) domain (Fig 2). It is not known quantitatively what percent of DNA damage in the cells is bypassed in a Rev1-dependent versus REV1-independent manner. This knowledge will help decipher whether a single inhibitor targeting the Rev1/RIR or the REV1/REV7 interaction or a combination of inhibitors targeting the REV1/RIR, REV1/Rev7 and UBM/UBZ-PIP-PCNA

interactions would be required for a complete TLS inhibition. Also, a better understanding of synthetic lethal partners of TLS polymerases would provide insights into which tumors might be most susceptible to chemotherapy treatments involving small molecule inhibitors of TLS polymerases. Finally, the effectiveness of small molecule inhibitors of TLS polymerase could be further improved by delivery systems that could target these drugs to specific tumors in cancer patients. Because protein-protein interactions are so important for TLS, drug targets for these interaction interfaces could be promising candidates for cancer therapeutics.

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