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Translesion Synthesis: An emerging new target during chemotherapy

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Abstract
Translesion synthesis (TLS) is an important mechanism where a group of polymerases come together to orchestrate the bypass of aberrant DNA lesions. This process enables survival of those cells, which would have otherwise been committed to die because of these difficult to repair DNA lesions. Interestingly, siRNA knockdown of TLS polymerases in cancer cells was shown to sensitize cells to increased killing and reduced mutagenesis following treatment with chemotherapeutic drugs, which suggests an important role of TLS polymerases in chemoresistance and cancer relapse. Recent investigations showed that small molecule inhibitors (SMi) that specifically bind REV1 TLS polymerases and suppressed its protein-protein interactions with other TLS polymerases, also successfully sensitized cancer cells to enhanced killing and reduced mutagenesis. As such, the possibility of a SMi that specifically binds REV1 and inhibits TLS, will serve as a chemotherapy adjuvant and prevent chemoresistance and cancer relapse is now a promising possibility. In this mini-review, we will provide a brief discourse of the overarching importance of TLS polymerases in normal and cancer cells and describe characteristics of current and future TLS inhibitors that will potentially serve as clinical chemotherapeutic adjuvants.
Key words: Translesion synthesis (TLS), TLS polymerases, Small molecule inhibitors (SMi), chemoresistance, cancer.

Translesion synthesis (TLS): the DNA damage bypass process

TLS is a highly conserved DNA damage bypass process whereby a group of specialized DNA polymerases replicate past aberrant DNA lesions. The translesion synthesis process is either error prone or error free depending upon whether an incorrect or a correct nucleotide was inserted across the damage. Both the structural attributes of TLS polymerases and the context of the DNA damage determines the choice of the nucleotide that is inserted across the damage [1, 2]. The consequence of an incorrectly inserted nucleotide by the TLS machinery is the introduction of mutation in the next round of replication [3]. The expense of this error-prone synthesis by TLS polymerases is to rescue cells from replication stress, which would have otherwise committed cells to cell-death from the un-repaired DNA lesion. Evolutionarily, the introduction of new mutations drives organismal fitness, but the same principal of inadvertent introduction of deleterious mutations propels tumorigenesis and disease [3, 4].

There are 10 human TLS polymerases—REV1, POL η, POL ι, POL κ, POL ζ, POL μ, POL λ, POL β, POL ν, and POL θ—distributed in 4 families (the Y, B, X and A) [3]. Prim Pol TLS polymerase, a TLS polymerase has an additional primase activity [4, 5]. In this brief review, we will focus on the Y- family TLS polymerases (REV1, POL η, POL ι, POL κ) and POL ζ of the B-family. Distinct structural characteristics of TLS polymerases aid their DNA damage bypass capability over the replicative polymerases. For instance, the TLS polymerases possess relatively smaller thumb and finger domains, which allow the formation of a

larger catalytic site to not only accommodate bulky damages, but also host the incoming nucleotide across it [1, 6]. In addition, the TLS polymerases lack the 3′-5′ exonuclease activity of replicative polymerases, thereby accepting the incorporation of incorrect nucleotides and facilitating an error-prone mode of DNA synthesis [7, 8]. The significance of this unique damage bypass ability and its importance to human health is seen in Xeroderma-Pigmentosum patients, who lack POL η, and are thus incapable of bypassing UV dimers regularly formed from sunlight and hence are cancer-prone [3, 9-11].

Mechanistically, the TLS process is proposed to proceed via two model pathways—the polymerase switch model and the gap-filling model [1, 3]. In the polymerase switch model, REV1 TLS polymerase functions as a scaffolding protein and orchestrates the 'insertion' across and 'extension' past the damaged DNA, whereby, 'insertion' polymerases, such as POL η, POL ι, POL κ, and less often REV1 itself (by means of its deoxycytidyl transferase activity), inserts nucleotides across the damaged base [12-14]. To extend DNA synthesis beyond the damaged site, POL ζ and in certain contexts, POL κ is used [12, 15]. In both instances of insertion and extension, REV1 exclusively utilizes it C-terminal domain (CTD), where distinct interfaces at the REV1 CTD are known to interact with both the insertion and the extension polymerases [16, 17]. REV1 CTD, a 100 amino acid, conserved domain, consisting of 4 amphipathic helices, engages in protein-protein interactions with both the inserter and extender polymerases [18]. Here, the REV1-interacting region (RIR) containing sequences of the inserter polymerases—POL η, POL ι and POL κ—contains two conserved phenylalanine residues that interact with specific residues within the α1 and α2 helix of REV1 CTD, forming one distinct RIR-interaction interface [17]. Similarly, several key residues within the α3 and α4 helices interact with REV7 of the POL ζ complex to form the other independent interface. REV1 CTD can simultaneously interact with both the inserter and extender TLS polymerases via these interfaces. It is speculated that the switch from insertion to extension is facilitated by the POL δ3 subunit of the POL ζ complex—(REV3, REV7, POL δ2 and POL δ3), which is now referred to as POL ζ_4 complex—which interacts with the REV1 CTD by its RIR sequences [19, 20].

The gap-filling model of TLS synthesis is proposed to account for several key cellular events, such as the immunoglobulin gene hypermutation, the final DNA synthesis step of the DNA repair pathways, and the filling of gaps across UV photoproducts [6, 21-23]. The exact nature of events that recruit the TLS polymerases and facilitate TLS synthesis at DNA gaps is largely less well understood.

More recently, TLS polymerases are known to play other important roles in cells, such as REV1-mediated replication of G-quadruplex structures and REV7-dependent cell cycle regulation and telomere maintenance [24-26]. Interestingly, TLS polymerases were also shown to contribute to chemoresistance during cancer treatment as is discussed next.

TLS-dependent chemoresistance and cancer relapse

Recently, three landmark articles revealed that siRNA-mediated suppression of TLS polymerases not only sensitized cancer cells to increased cell-death, but also reduced their acquisition of intrinsic and acquired mutations during chemotherapy [4]. These observations suggest that the TLS polymerases modulate tumor response to

chemotherapy [4]. The first of these three articles showed that Rev3 inhibition in the lung adenocarcinoma cells, $K^{rasG12D}/p53^{-/-}$, not only sensitized these cells to cisplatin treatment, but also enhanced survival of these Rev3 deficient tumors [27]. Even the cisplatin-induced mutagenesis in these $K^{rasG12D}/p53^{-/-}$ cells was reduced after knockdown of either Rev3 or Rev1. Likewise, by using the *Eμ-myc arf*$^{-/-}$ mouse model of B-cell lymphoma, it was shown that recurrent cycles of engraftment of Rev1-deficient tumors along with cyclophosphamide treatment, exclusively sensitized these tumor cells to chemotherapy compared to the Rev1-proficient engrafted tumors [28]. Similar to the lung adenocarcinoma cells, the B-cell lymphoma cells had reduced cyclophosphamide-induced mutagenesis, which strongly supports the hypothesis that TLS polymerases regulate chemotherapy responses in cancer cells and that they play an important role in acquired mutagenesis. This is an important observation given the fact that tumor relapse is known to be caused by chemotherapy-induced mutagenesis. In the third breakthrough publication that explored an siRNA mediated strategy to knockdown both Rev1 and Rev3 via a nanoparticle-mediated delivery system showed a dramatic inhibition of tumor growth and enhanced survival in the LnCaP prostate xenograft mouse model [29].

Subsequent studies, such as the REV7 depletion in ovarian cancer cells; REV3 inhibition in cervical cancer cells; etc., also showed an enhanced sensitization of cancer cells to chemotherapy and reduction in mutagenesis—thereby augmenting strong support to the hypothesis that TLS polymerases modulate tumor response to chemotherapy and mutagenesis [30, 31]. Together, these studies provide compelling evidence that by directly inhibiting the TLS polymerases in cancer cells by drug targets, cancer cells can be similarly sensitized to enhanced killing and reduced mutagenesis.

Drug targets to suppress TLS

In order to successfully inhibit TLS, conceptually, both the catalytic activity and the key protein-protein interactions of the TLS polymerase can be targeted. Molecules that would disrupt these activities can be used as adjuvants during chemotherapy. Examples of molecules that specifically target the catalytic function of TLS polymerases include, pamoic acid, aurintricarboxylic acid, and ellagic acid to inhibit POL ι and POL η's; candesartan cilexetil to target POL κ; and 3-O – methylfunicone that obstructs POL κ, POL, i, POL η activity [32-34]. Although compelling in extent of a specific TLS polymerase inhibition, the catalytic function inhibitors may not be sufficient to suppress TLS completely. Often the TLS polymerases exhibit redundant functionality, whereby absence of one TLS polymerase is compensated for by another TLS polymerase to rescue the ensuing replication stress. For example, POL ι and POL κ can bypass UV-dimers in the absence of POL η [11, 35], which would necessitate the use of multiple inhibitors. Moreover, more work needs to be done to ascertain their target specificity and potency as drug targets *in vivo*.

On the other hand, the second avenue of inhibiting TLS by targeting the protein-protein interactions could be a promising strategy. So far only two examples exist that illustrate the possibility of targeting the protein-protein interactions of TLS polymerases. In the first instance, a REV7 binding small molecule inhibitor that disrupts its interaction with REV3, was used to moderately suppress interstrand cross link repair in cells [36]. Whether this same inhibitor could suppress TLS is not known. In the second instance, two small molecule

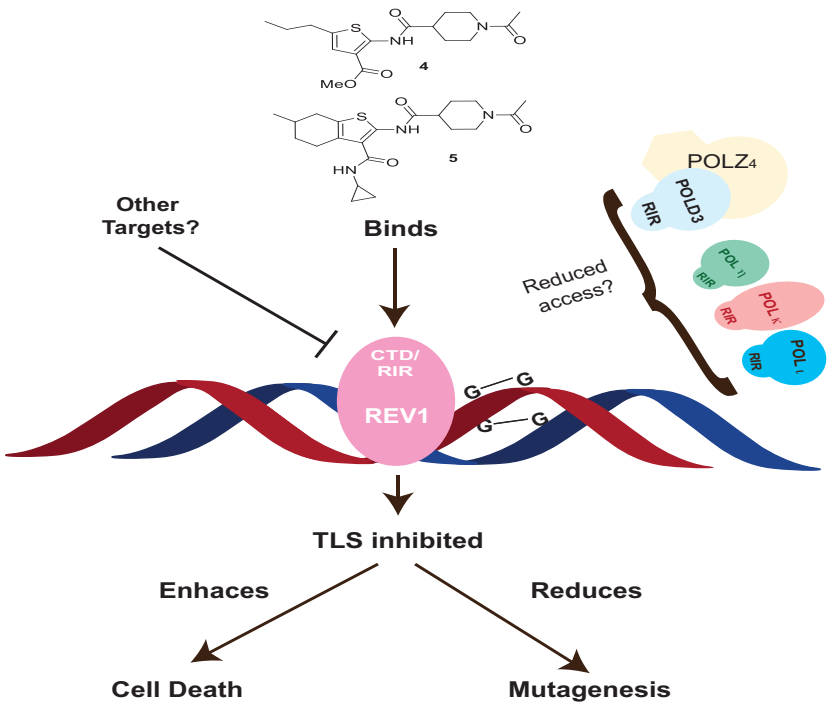
inhibitors (SMi) that targeted the RIR interface of REV1 were shown to inhibit TLS [37]. Here, the two molecules coined 4 and 5 were shown to first bind the RIR region of REV1 on specified residues in a fluorescence polarization (FP) assay. Further, these SMi were shown to enhance the cytotoxicity of cisplatin-treated cells, while reducing mutagenesis rate at the *HPRT* locus. This is the first true example of TLS inhibitors that have the potential to be tested as adjuvants during chemotherapy treatment in patients (Figure 1).

Being the first study to characterize a promising TLS adjuvant, key observations from this study are briefly described here. The SMi 4 and 5 were identified in a FP pilot screen of 4800 molecules. These small molecules or scaffolds contain a "right-side" piperidinyl ketone linked across an amide bond to a "left-side" substituted thiophene (Figure 1). Nuclear magnetic resonance (NMR) studies revealed a direct binding of these molecules to the REV1 CTD as indicated by the shift in the NMR spectra. Further, incubation of mouse and human cells with cispatin and the SMi's resulted in enhanced killing, while reducing the *HPRT* gene mutation rate in these cells. Whether, these molecules can reduce tumor volume and enhance survival in cancer mouse models in not known. These experiments would be key to discovering drug targets to combat chemoresistance in patients.

Conclusion

Translesion synthesis (TLS) is an important DNA damage bypass process, which allows replication to continue in the presence of aberrant DNA lesions. Recent reports indicate that knockdown of key TLS polymerases sensitizes cancer cells to enhanced killing, while also reducing their acquired mutagenesis rates. These observations suggest that TLS polymerases could be responsible for the chemoresistance and cancer relapse seen in patients. As such, inhibiting TLS polymerase activity during chemotherapy could be an effective strategy for cancer treatment. Very recently, small molecule inhibitors (SMi) have been shown to bind specific REV1 interfaces *in vitro* and sensitize cisplatin-treated cells, while reducing their mutagenesis rate. Future studies that would show the efficacy of these SMi in reducing tumor volumes and enhancing life spans in tumor mouse models would help establish TLS inhibitors as promising adjuvants during chemotherapy treatment.

Figure 1: Inhibition of translesion synthesis by targeting REV1-mediated protein-protein interactions by molecules that binds to its distinct interfaces. Shown are two exemplary molecules 4 and 5 that target the RIR interface of REV1, which could reduce access to the RIR containing TLS polymerases and inhibit TLS. The read outs of an inhibited TLS would be an enhanced cell death and resuced mutagenesis. "Adapted with permission from (Identification of Small Molecule Translesion Synthesis Inhibitors That Target the Rev1-CT/RIR Protein−Protein Interaction. Vibhavari Sail, Alessandro A. Rizzo, Nimrat Chatterjee, Radha C. Dash, Zuleyha Ozen, Graham C. Walker, Dmitry M. Korzhnev, and M. Kyle Hadden. *ACS Chemical Biology* **2017 *12* (7), 1903-1912. DOI: 10.1021/acschembio.6b01144).** Copyright (2017) American Chemical Society".



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