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Multifaceted functionality of Thymine DNA Glycosylase (TDG) in tumorigenesis: implications regarding efficacy of TDG-based cancer therapy

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Abstract

The continual threat cancer poses to human health coupled with the worrisome frequency of tumor resistance to existing drug-based therapies necessitates the development of new intervention strategies. Thymine DNA Glycosylase (TDG), canonically associated with excision of specific nitrogenous DNA bases in the intranuclear processes of Base Excision Repair and active DNA demethylation, has been suggested as a novel target for therapeutic intervention due to its dual role in the maintenance of both genomic and epigenomic stability. This review aims to clarify how various genomic functions of TDG may contribute to the onset or progression of tumorigenesis, in addition to the significance of TDG function as it relates to TDG-based cancer intervention. The observed role of TDG in cancer development is highly context-dependent, with TDG either promoting or inhibiting tumorigenic processes based on factors such as cancer type. TDG also mediates expression of several key oncogenes and tumor suppressors via transcriptional regulation at gene promoters as well as physical interaction with protein products, suggestive of its indirect regulatory role in tumorigenesis. Aberrant rates of TDG-mediated processes may additionally influence efficacy of existing cancer therapies through mechanisms such as promotion of radioresistance or alteration to apoptotic signaling pathways. Importantly, alteration of TDG expression during cancer intervention may potentiate unintended genomic or epigenomic intracellular effects due to TDG moonlighting, with TDG primarily functioning in BER and active DNA demethylation but also regulating expression of several physiologically relevant proteins. Chimeric technology including PROTAC could be utilized to normalize TDG concentration – regardless of differential over or under-expression - in tumor tissue wherein TDG is abnormally expressed.

Keywords

Thymine DNA Glycosylase, TDG, Tumorigenesis, Base Excision Repair, DNA demethylation, Cancer intervention, Oncogene, Tumor suppressor, Proteolysis Targeting Chimera, Ubiquitin

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1. Introduction

Despite increasing amount of research into cancer cell propagation and proliferation, current understanding of the genetic and epigenetic factors contributing to the onset of tumorigenesis remains incomplete. Aberrant chemical modification, including deamination and oxidation of DNA nucleotides, threaten genomic integrity via damage to the intricate nucleome structure, and without the occurrence of sufficient reparation, may result in mutations that induce tumorigenesis (1, 2). Tumorigenesis onset initially requires multiple critical mutations to circumvent induction of apoptosis or DNA repair mechanisms, with the specific amount required to do so estimated at approximately three to seven mutated nucleotide bases (2). Most frequently, genetic mutations appearing within cancer cells result from cytosine to thymine lesions in somatic cell DNA, occurring in the context of CpG dinucleotides (3, 4, 5). This incidence can be attributed to the non-enzymatic (spontaneous) deamination of 5-methylcytosine (5mC) into thymine, resulting in a G:T mispair, which is typically corrected via the Base Excision Repair (BER) metabolic pathway (4). ~ 20,000 potentially mutagenic or cytotoxic DNA lesions are generated every day in each human cell by reactive oxygen species, with BER serving as the predominant mechanism for the repair of these lesions and prevention of associated potentially damaging effects (6). The initial phase of BER involves excision of the mismatched base by a DNA Glycosylase enzyme, such as Thymine DNA Glycosylase (TDG), resulting in an abasic (AB) site, which is subsequently cleaved from the DNA strand by Apurinic/Apyrimidinic Endonuclease 1 (APE1) and, in series of reactions involving DNA Polymerase β and DNA ligase, replaced with the appropriate nucleotide (Figure 1). In the context of the G:T lesion, TDG performs the excision of thymine to initiate the BER process. Alterations to this critical function of TDG are thought to be associated with tumorigenesis due to an associated increase in mutagenic potential, especially given the commonality of cytosine to thymine mutations in cancer.

Broadly, TDG has been implicated in several genetic and epigenetic processes, including DNA repair, DNA demethylation, and transcriptional regulation. TDG is a part of the Mismatch Uracil Glycosylase branch of the mammalian Uracil DNA Glycosylase superfamily, characterized by a common α/β structure and the presence of a highly conserved central catalytic domain connected at either end to a less conserved N-terminal or C-terminal domain (7, 8). In humans, the gene coding for TDG is composed of 10 exons on chromosome 12q24.1, covering in total 23 kiloBase pairs (9). The TDG enzyme itself consists of 410 amino acid residues, with residues 123 to 300 comprising a central catalytic domain regulating glycosylase functionality (10). The N-terminal (Residues 1 to 111) and C-terminal (Residues 328 to 410) domains of TDG contain lysine-rich regulatory regions, mediating enzymatic functioning and key molecular interactions (8).

Cytosine methylation, spontaneous deamination of 5mC to thymine, and, albeit less frequently, deamination of cytosine to uracil are frequently observed in the context of

CpG islands, generating G:T and G:U DNA lesions respectively. Located near to or within approximately half of all mammalian DNA promoter regions as well as within certain enhancer, intergenic, and intronic DNA regions, CpG islands consist generally of 500 to 3000 base pairs and include an exceptionally high frequency of CpG dinucleotides (8). It is known that CpG islands, typically hypomethylated in undamaged cell promoter regions and indicative of transcriptionally active genes, are associated with transcription of affiliated genes (12, 13). Moreover, aberrant methylation patterns in CpG islands have been characterized as an "epigenetic hallmark" of the cancerous genome and can arise from inhibition or deactivation of methylation pathways (12). Typically, in cancer, hypermethylation of promoter CpG islands is associated with silencing of tumor suppressors, whereas hypomethylation is associated with overexpression of oncogenes.

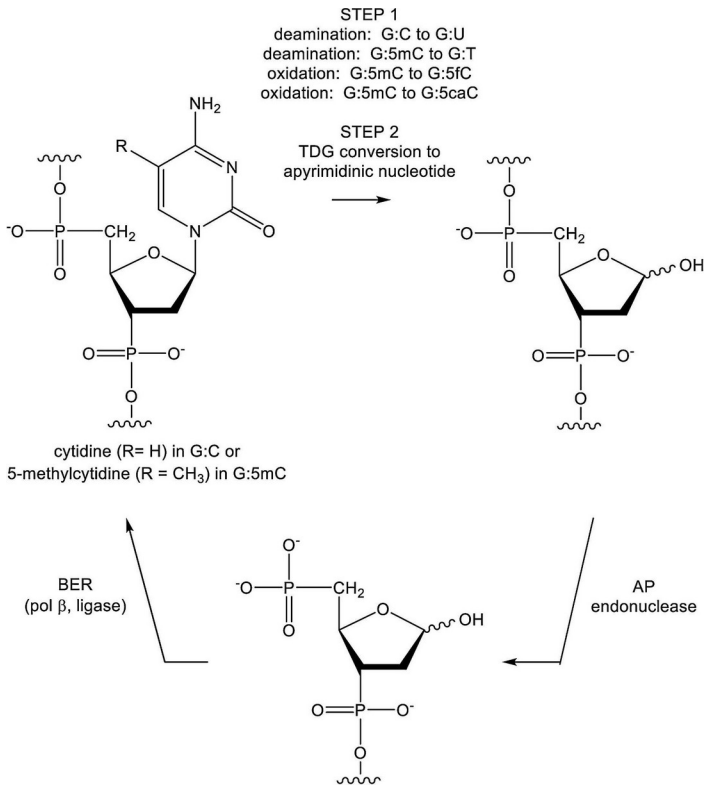

Figure 1. Steps of BER are shown. Chemical modification of a DNA nucleobase, depicted in Figure 1 as either C or 5mC, results in a mismatch lesion, which is excised by TDG to generate an AB site. APE1 is recruited by TDG to cleave the AB site from the DNA strand, and DNA Polymerase β works with DNA ligase to insert the appropriate nucleotide and repair the DNA strand to complete the BER pathway. (Figure adapted from Figure 2 in Xu et al. 2016: Reference 9 in this paper).

DNA Methyltransferases (DNMTs) including DNMT1, DNMT3A, and DNMT3B catalyze the addition of a methyl (-CH_3) group to a cytosine base, generating 5mC to influence regulatory behaviors at the CpG islands (12). DNMT3A and DNMT3B generate 5mC in a *de*

novo manner, utilizing methyl donor S-Adenosylmethionine (SAM) as a source from which the methyl group is transferred to the C5 carbon of cytosine, whereas DNMT1 works with cofactor Ubiquitin-like Containing Plant HomeoDomain (PHD) and Really Interesting New Gene (RING) Finger Domains 1 (UHRF1) to ensure correct reproduction of 5mC during DNA replication in cell division (8). Demethylation of DNA is initiated by Ten Eleven Translocation (TET) enzymes in a sequence of iterative reactions that oxidize 5mC to 5-hydroxymethylcytosine (5hmC); then to 5-formylcytosine (5fC), and finally to 5-carboxylcytosine (5caC) (8). TDG catalyzes the excision of 5fC or 5caC, but not 5mC or 5hmC, in a process similar to BER, generating an AB site which is subsequently repaired via the insertion of an unmodified cytosine. Recent research suggests that oxidized 5mC derivatives possess distinct functionality in the regulation of various pathological and physiological processes to maintain cell homeostasis, such as transcriptional inhibition performed by 5mC and 5hmC or alteration, primarily repression, of promoter activity performed by 5fC and 5caC (13, 14).

DNA methylation facilitates genomic stability and imprinting, influences gene expression, controls X chromosome inactivation, generates silencing of transposons, alters chromatin accessibility, and regulates the establishment of pluripotency during embryogenesis (12, 15, 20). DNA demethylation involving TDG activity occurs via two distinct means: passive and active demethylation. Passive demethylation occurs when, due to the rendering of DNMT1 to a nonfunctional state either by inhibition, nuclear exclusion, or deactivation of the enzyme itself or a critical cofactor (i.e. UHRF1), DNA methylation gradually decreases over multiple periods of replication during cell division (8). Contrastingly, active demethylation occurs independently of the cell cycle via endogenous enzymatic activity, such as that of the TET-TDG demethylation pathway (21).

Certain covalent modifications occur specifically at either TDG's N-terminus or C-terminus, differentially affecting TDG behavior in its various pathways. The polycationic N-terminal domain of TDG can undergo either acetylation or phosphorylation in a mutually exclusive manner, whereas the C-terminal domain undergoes sumoylation, the covalent addition of Small Ubiquitin-like Modifier (SUMO) (9). Acetylation has been observed specifically to occur at lysine residues 59, 83, 84, and 87 (11), with acetylation inhibiting TDG excision activity in G:T, 5fC:G and 5caC:G contexts but promoting more frequent TDG-mediated excision in the context of 5-fluorouracil (5flU):G (16). Importantly, 5flU is a relatively common chemotherapeutic drug due to its ability to induce cytotoxicity, with increased TDG expression associated with increased sensitivity to the drug whereas TDG depletion has been shown to confer resistance (17). Thus, TDG acetylation may be correlated to increased efficacy of 5flU chemotherapy treatment, as increased TDG excision activity at 5flU:G due to acetylation may mimic the effects of increased TDG excision activity in general due to overexpression. Phosphorylation occurs at TDG serine residues adjacent to acetyl-acceptor lysine residues (18). Although

phosphorylation has no direct impact on TDG interaction with DNA, it may preserve TDG functionality in G:T, 5fC:G and 5caC:G pairing by inhibiting acetylation at acetyl-acceptor lysines (18). Sumoylation occurs at TDG lysine residue 330, found within a sumoylation consensus motif (VKEE) at TDG's C-terminus. Sumoylation has been observed in one study to promote BER activity at G:U mispairs, but suppress TDG identification of G:T in the genome (7). In a separate investigation, sumoylation of TDG reduced substrate affinity in G:T, G:U, G:fC, and G:caC contexts, lessening BER activity at these pairings (7). Further research is needed to clarify the impact of TDG sumoylation on glycosylase activity in certain nucleobase pairs. Additionally, SUMO conjugation to the TDG C-terminus severely impairs the stability of TDG binding to AB sites and prevents further TDG-SUMO interaction, but increases the enzymatic turnover of bound TDG in BER by promoting TDG-mediated recruitment of APE1 to the AB site (7). Sumoylation may also prevent TDG acetylation by inhibiting TDG interaction with histone acetyltransferase Cyclic AMP Response Element-Binding protein (CREB) Binding Protein (CBP) (18).

Additionally, ubiquitination of TDG occurs in a Cullin-RING Ligase 4 ($CRL4^{Cdt2}$) ubiquitin ligase dependent manner prior to the cellular S phase to target TDG for degradation via a proteasomal pathway. Ubiquitination is crucial to prevent 5flU-associated cytotoxicity due to accumulation of excess TDG (19).

TDG plays a critical direct role in DNA repair, DNA demethylation, and transcriptional regulation, safeguarding the integrity of both the genome and the epigenome. Dysregulation of these TDG-mediated processes can allow for the bypassing of cell cycle checkpoints and uncontrolled proliferation that characterizes cancer development. Additionally, aberrant TDG expression has been observed in numerous types of cancer, suggesting a role of TDG in the establishment of the cancerous genomic landscape. Ultimately, this paper aims to answer the questions: *What are the multifaceted functions of thymine DNA glycosylase (TDG) in human tumorigenesis and how can this insight be used to develop efficacious TDG-based cancer therapies?*

2. Methods

The aim of this literature review is to determine how alterations to TDG structure or expression impact the progression of tumorigenesis. Relevant literature was identified via the PubMed and Google Scholar databases, utilizing general keywords such as "TDG", "Base Excision Repair", "Active DNA Demethylation", "Tumorigenesis", "Cancer Treatment", and "Pluripotency". Results were then filtered to include solely articles with the full text freely available, as well as being published within the past 10 years to establish currency of information. The remaining articles were then assessed for credibility and relevance of content, utilizing factors such as peer-review status and reauthor affiliation, before inclusion in this review. A second round of source identification was then conducted with the aim of clarifying emergent knowledge gaps using specific keywords such as the names of certain enzymes or transcription factors. During this process, articles with older publication dates

were also considered, with preference being given to more recently authored works.

3. Discussion

3.1 Molecular functions of TDG

3.1.1 *Base Excision Repair*

TDG catalyzes the excision of several purine and pyrimidine bases from mispairs in the BER pathway. To circumvent unwarranted glycosylase activity on undamaged DNA, substrate specificity is essential in TDG function (22). Most notably, TDG excises T or U from U:G and T:G mispairs (8, 9). TDG has additionally been found to excise oxidized bases and halogenated uracil derivatives thymine glycol, 5-formyluracil (5foU), 5flU, 5-chlorouracil, 5-hydroxyuracil, 5-hydroxymethyluracil, 3, N^4-ethenocytosine, 5-hydroxycytosine, 7,8-dihydro-8-oxoadenine, and 5-bromocytosine from DNA (8). Recently, TDG was also implicated in the excision of oxidized adenine (oxoA) from G:oxoA, A:oxoA, C:oxoA, and T:oxoA pairings (23). Compared to noted pyrimidine bases, oxoA excision has been noted to occur at a remarkably rapid rate (18- to 480-fold faster) in the context of G:oxoA (23). Although to a lesser extent, the rate of excision for C:oxoA and A:oxoA also surpasses that of the pyrimidine bases (23). Finally, TDG has been observed to remove T from A:T pairings in the context of damaged adenine bases (9), as well as from mispairings with 8-bromoguanine, which may induce mutation (primarily G to T mutation) if not corrected (24). Additionally, TDG catalyzes the excision of 5-carboxyluracil (5caU) as well as 5foU from 5caU/5foU:G mispairs (1). Interestingly, 5caU serves as one of the comparatively best substrates of human TDG, significantly better than thymine (1).

The involvement of TDG in the BER process can be organized into a cyclical pathway distinct from the overall BER pathway, involving the following sequential steps: (1) identification of the target nucleobase, (2) extrahelical flippage of the target, (3) cleavage of the N-glycosidic bond to separate the target from the DNA strand, and finally (4) release of the excised base followed by TDG dissociation from the DNA (25). To efficiently search the DNA sequence for lesions, TDG employs a variety of linear diffusion mechanisms including “hopping” and “sliding” across the strand (26). Sliding activity in an associative manner has been noted in the function of Uracil DNA Glycosylase as well (25). TDG has additionally been proposed to utilize 3D random collision and intersegmental transfer methods to augment its efficacy in lesion identification (25) (Figure 2). Intriguingly, it has been observed that TDG translocation, often attributed to electrostatic interactions between TDG and DNA, occurs at a faster rate when TDG is located at a greater distance from DNA lesions, and ultimately slows as TDG approaches its target site (27), although the specific mechanisms contributing to the differing speed of TDG translocation are not fully understood. Overall, the combination of these four major searching mechanisms allows for highly effective target searching given the context of genomic folding and crowding imposed by the cellular environment (25).

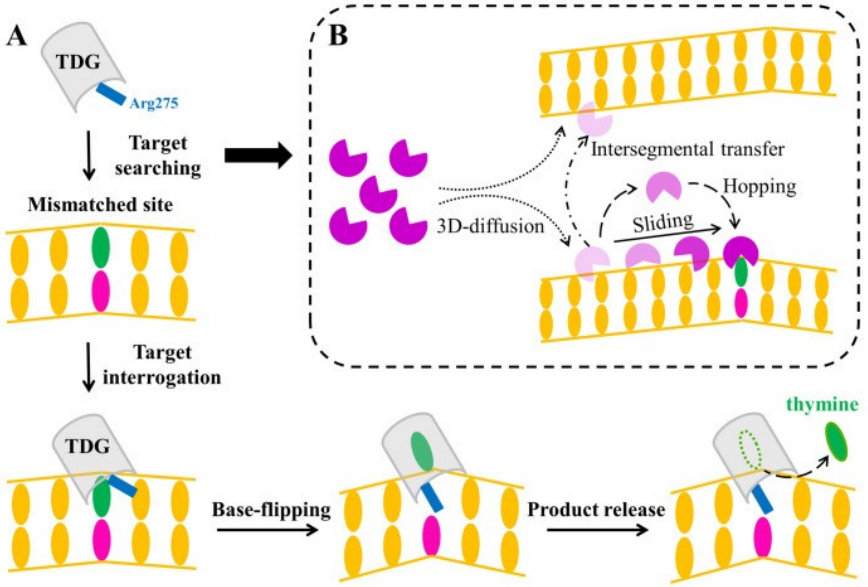

Figure 2: Processes involved in TDG-mediated base excision are depicted. (A) TDG inserts an arginine-containing loop (Arg275) into the DNA minor groove at the site of a mismatch lesion to transiently disrupt base pairing and facilitate highly stochastic base interrogatory mechanisms, followed by extrahelical flippage of the target DNA nucleobase (shown in the figure as thymine), cleavage and ultimately release of the excised nucleobase into the intranuclear environment. (B) TDG employs several distinct mechanisms of DNA translocation to effectively search for its target, including 3D diffusion, intersegmental transfer, and short-range "sliding and hopping" (Figure adapted from Figure 1 in Wang et al. 2022: Reference 25 in this paper).

TDG detects lesions via the insertion of a loop containing arginine into the DNA minor groove, momentarily separating nucleotide pairings to allow for a stochastic base interrogation (28). Additionally, enzyme kinetics and interrogatory mechanisms have been correlated to the equilibrium state of the DNA phosphate backbone, suggesting that the DNA structure itself may function in the identification of mispairs or the flipping of nucleotides during BER (29). Furthering this observation, Westwood et al. propose for G:T contexts specifically that perturbed DNA backbone energetics allow for mispair identification by DNA repair enzymes (30). TDG binds to DNA at the lesion site through utilization of its N-terminal domain as a "flexible clamp" (9) and catalyzes the extrusion of the target nucleotide from the DNA helix, binding it instead to the TDG active site (31). Then, TDG facilitates the cleavage of the N-glycosidic linkage of the target nucleobase to the pentose sugar by utilizing a nucleophilic water molecule to attack the anomeric carbon C1′, forming an AB site (25). Release of the excised base is critical to liberate TDG for subsequent regulatory and enzymatic activity (25). TDG then binds via its N-terminus at the AB site with an exceptionally high affinity and protects the unstable AB region from

degradation before it can be enzymatically repaired (9). The role of TDG in BER completes with the conjugation of SUMO-1 at the C-terminal domain of TDG, which helps facilitate the recruitment of APE1 to bind at TDG residues 92 to 121 and catalyze the dissociation of TDG from the AB site (9), allowing TDG to resume processes associated with target-searching and lesion identification. APE1 then attracts DNA Polymerase to insert the correct nucleotide base and repair the AB DNA with the assistance of DNA Ligase, completing the BER pathway.

Importantly, DNA arrangement into nucleosomes may alter enzymatic accessibility during lesion repair-associated processes, and certain factors, such as ion concentration, contributing to the cellular environment and microenvironment, may influence DNA repair enzyme recognition of lesions as well as efficiency of DNA repair pathways (27). Thus, modification of epigenetic factors may be utilized to enhance or reduce TDG excision activity in the genome, promoting or repressing its effects.

3.1.2 *DNA demethylation*

TDG recognizes and selectively excises TET-oxidized 5mC derivatives 5fC and 5caC, but not 5mC or 5hmC, from DNA, demonstrating its cruciality to, and specificity in, the active DNA demethylation pathway. To explain this specificity in TDG excision activity, a mechanism has been proposed by which N_3 acidity is augmented by the electron-withdrawing behavior of the -CHO or -COOH attached to 5fC or 5caC respectively, disrupting hydrogen bonds critical to base pair stability, allowing for a highly selective recognition by TDG (32). Moreover, Pidugu et al. observe that asparagine residue N191, implicated in the excision of 5caC, makes direct contact with N_3 and N_4 of 5caC during excision, further suggesting the role of acidity in catalysis of this highly selective process (33). Additionally, the integration of formyl- and carboxyl- functional groups have been shown to induce geometric alteration to the DNA minor groove which in turn are recognized specifically by the R275 finger residue of TDG (34), suggesting an alternative mechanism behind TDG selective recognition of 5fC and 5caC. In terms of base pairing, excision rates of 5fC and 5caC are only marginally dependent on context, with only a slightly faster rate of activity at CpG sites relative to CpA sites, which starkly contrasts the highly context dependent excision rates in the canonical BER pathway (35). Although the specific nucleobase pair at which methylation occurs appears not to affect rates of TET-TDG demethylation activity, the relative genomic location of the oxidized 5mC derivative may influence the frequency of demethylation activity. TDG depletion experiments reveal, based on alterations to the genomic formylation landscape, that active demethylation via TDG occurs most frequently at intron/exon boundaries, particularly in the context of CpG islands (36).

Under deactivation of the BER pathway, accumulation of 5caC but not 5fC in CpG islands represses promoter activity, suggesting a role of 5caC in the inhibition of premature promoter activation during the final steps of DNA demethylation (37). Additionally, 5caC has been observed to accumulate during

differentiation of neural stem cells (38), linking active DNA demethylation to development and lineage specification processes. 5fC has been implicated in embryonic development due to enriched levels of 5fC observed in active enhancer regions of DNA, allowing for specific tissue-diagnostic patterns to develop (36). It is important to note that the 5fC distribution in embryogenesis parallels that of Embryonic Stem Cells (ESCs), both having relatively higher levels of 5fC in active enhancer and intragenic regions, suggesting a role of DNA methylation status in the establishment of the pluripotent state (36). Moreover, under TDG depletion, promoter regions of ESCs identified to become methylated during differentiation underwent the largest increase in 5fC presence, demonstrating the importance of 5fC excision to establish correct genomic methylation patterns (8). Both 5fC and 5caC levels increase proportionally with the accessibility of gene promoter regions in ESCs, potentially contributing to the observed genomic localization of TET and TDG occurring simultaneously to this phenomenon (8) and further connecting presence of TET-oxidized 5mC derivatives to embryonic development. Additionally, hemimethylated promoters including either single 5fC or 5caC metabolites were observed to undergo increasingly reduced activity in a TDG-dependent manner, ultimately being rendered to a state of significant repression (14), associating active DNA demethylation to an increase in gene expression. In general, increased 5fC and 5caC presence is observed at active promoter, active enhancer, and exon DNA regions with the H3K4me3 chromatin mark (8), furthering the link between the TET-TDG active DNA demethylation pathway, 5fC/5caC metabolites, and gene expression. Potentially, this link could be exploited to intentionally modify gene expression in human cancer, providing a conceptual basis for TDG-based intervention.

Slyvka et al. also confirmed the direct binding of TDG to Nei-like 1 (NEIL1), another enzyme implicated in direct excision of 5fC and 5caC during the active DNA demethylation pathway, and observed NEIL1 activity in the 2'-deoxyribose excision step of demethylation occurring downstream of TDG excision. NEIL1 was also demonstrated to increase TDG-mediated genomic excision of 5fC and 5caC in a substrate-dependent manner (39). Thus, NEIL1 may be targeted to selectively alter TDG activity for certain substrates (i.e. 5fC and 5caC) but not others.

Cohey and Drohat suggested that sumoylation of TDG may temporarily enable TDG functionality as a reader of 5fC and 5caC nucleobases and enhance TDG ability to recruit other enzymes or transcription factors to 5fC or 5caC sites (40). However, a separate study determined that TDG-mediated excision of 5caC was not dependent on sumoylation (41), demonstrating some uncertainty over the role of sumoylation in active DNA demethylation processes.

TDG additionally plays a marginal role in passive demethylation via its interaction with DNMT3A, inhibiting DNMT3A function and thus preventing the occurrence of *de novo* DNA methylation (9).

3.1.3 *Transcriptional regulation and chromatin remodeling*

TDG functions in the epigenetic modulation of chromatin structure, influencing expression of associated genes. TDG induces the decondensation of chromatin fibers (8) via physical interaction with linker DNA, allowing for increased access of enzymes or transcription factors to unwound DNA compared to DNA in a highly condensed state. Moreover, chemically defined nucleosome arrays found that TDG mediates chromatin condensation through its N-terminal domain, which is structurally reminiscent of the C-terminus of linker histone H1.1 (i.e. both possess low complexity, significant disorder, and highly basic components), implicated in chromatin condensation and secondary structural stabilization (42). After binding to DNA, TDG utilizes long-range fiber interactions to catalyze chromatin oligomerization into higher order structures (42). However, TDG's C-terminal domain antagonizes this process, promoting high levels of chromatin condensation in its absence or truncation (Figure 3) (42). TDG also demonstrated *in vitro*, the ability to induce DNA phase separation under physiological conditions, in a process dependent on its N-terminal and C-terminal domains (43). Separately, each domain catalyzes the formation of differentially propertied chromatin condensates, in alignment with the unique role played by each domain in the phase separation process (43).

DNA methylation influences TDG-mediated regulation of chromatin condensation status, suggesting an intrinsically meticulous and even self-induced regulatory mechanism of transcriptional activity via epigenetic alteration. Although TDG can bind at methylated DNA regions, methylation prevents the occurrence of TDG-mediated chromatin condensation (42), altering chromatin structure and associated transcriptional regulation. Further research is necessary to determine the mechanism whereby this process occurs. 5fC and 5caC accumulation have also been shown to epigenetically alter transcriptional activity via changes to DNA supercoiling and packaging through means such as increased DNA flexibility (8). Changes in TDG expression or genomic structure, commonly observed in tumor growth, may disrupt the balance of intranuclear processes critical to epigenomic integrity, further dysregulating transcriptional activity to facilitate the progress of cancer development.

Chemical modification of histones is partially dependent on TDG activity, utilizing feedback mechanisms for the maintenance of cellular homeostasis. TDG deficient mouse embryonic fibroblast cells exhibit a genome-wide reduction in histone acetylation rate (44). Histone acetyltransferase p300 binds TDG at its -CH_3 domain as an allosteric coactivator in the acetylation of lysine residues K18 and K23 of histone H3 (44). Contrastingly, TDG behaves as a competitive inhibitor of p300 when localized TDG levels approach histone concentrations (44). TDG-based regulation of histone modification additionally alters cell differentiation processes. Exemplifying this relationship, TDG activity has been demonstrated to prevent the oxidation of 5mC necessitated for histone modification

promoting myeloid cell differentiation to macrophages and osteoclasts (45).

TDG activity was implicated in embryogenesis, demonstrating key regulatory roles in cell-cycle dependent fate determination and transcriptional heterogeneity (46). In connection with the aforementioned role of TDG in histone alteration, TDG has been shown to mediate the synaptic activity and euchromatin formation during differentiation via interaction with active histone modification enzymes, including CBP and p300, in embryonic development (9). Further, the reprogramming of mouse embryonic fibroblast cells to induced pluripotent stem cells was inhibited in the absence of TDG (8), cell fate determination during early differentiation was altered in mouse embryonic stem cells under transient TDG knockout (46), and TDG depletion prevented differentiation of pig preadipocytes (47). Active DNA demethylation pathways through TDG also play a role in cell fate determination and embryonic development. DNA methylation influences expression of several key transcription factors, providing a mechanism by which fibroblast reprogramming to the pluripotent state is dependent on TDG demethylation activity (47). Intriguingly, TDG performs defensive behavior against aberrant methylation at CpG dinucleotides located in promoter regions associated with critical developmental genes (43), ensuring proper expression.

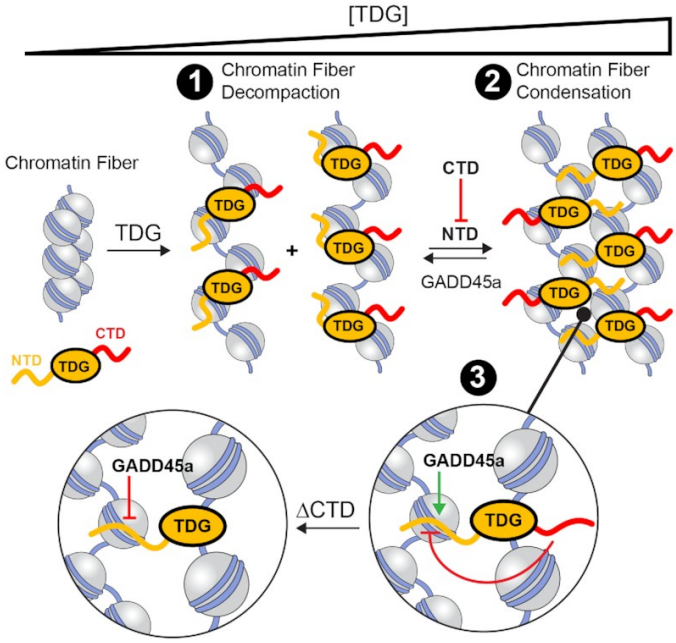

Figure 3. Proposed mechanism of TDG-mediated chromatin oligomerization by Deckard and Sczepanski. CTD refers to the C-terminal domain of TDG. NTD refers to the N-terminal domain of TDG (Figure adapted from Figure 6 in Deckard & Sczepanski 2021: Reference 42 in this paper).

Dysregulation of TDG-mediated transcriptional pathways threatens genomic stability. TDG binds several members of the nuclear receptor superfamily, including Retinoid Acid Receptor α (RARα), Retinoid X Receptor α (RXRα), androgen receptor, glucocorticoid receptor, progesterone receptor, vitamin D3 receptor, peroxisome proliferator-activated receptor, thyroid hormone receptor, and estrogen receptor (ER) (8). Due in some capacity to the dysregulation of the Retinoic Acid (RA) signalling pathway, TDG depletion proved to be embryonic lethal ~ at day E11.5, with mouse Embryonic Stem Cells (mESCs) also failing to undergo RA-dependent differentiation (8). Both of these findings indicate the importance of TDG function to the growth and vitality of the mouse embryo. Interestingly, despite the cruciality of TDG in active demethylation processes for mESCs and induced pluripotent stem cells, it has been shown that the role of TDG in pronuclear DNA demethylation is insignificant, and TDG expression is minimal in the mouse zygote (48).

3.2 TDG activity in tumorigenesis

3.2.1 *Key intermolecular interactions at TDG structural domains*

Interactions with several physiologically relevant enzymes and transcription factors occur distinctly at the N-terminal, central catalytic, or C-terminal domains of TDG, regulating protein functionality (Figure 4). Residues 1 to 111 comprise TDG's N-terminal domain, which binds TDG directly to DNA during base excision, as well as binding APE1, DNMT3A, Estrogen Receptor α (ERα), the Histone AcetylTransferase (HAT) domain of CBP/p300, Protein Kinase C α (PKCα), Sirtuin-1 (SIRT1), and Transcription Factor 4 (TCF4) (9, 10). Alteration to TDG expression or expression of any of the aforementioned proteins may in turn result in modification to TDG-mediated interactions, potentially disrupting or overpromoting key functions of any of the aforementioned proteins and damaging genome integrity. TDG interactions with APE1 and DNMT3A help to facilitate the processes of BER and active DNA demethylation, respectively (8). TDG represses the methylation-inducing ability of DNMT3A, but DNMT3A promotes excision activity of TDG (9), suggestive of a complex molecular relationship influencing DNA demethylation. Thus, alterations to TDG interactions with either APE1 or DNMT3A may cause dysregulation of either critical pathway, causing potential damage to genome integrity via uncorrected mutagenesis or epigenetically (i.e. abnormal methylation) induced disruption of transcriptional regulation.

To promote transcription of associated genes, CBP/p300 catalyzes histone H3 lysine 27 acetylation at promoter and enhancer regions (49). Although CBP/p300 depletion was historically identified to induce tumor development, research over the past several years has demonstrated that CBP/p300 is also overexpressed in many cancers, with high CBP/p300 expression associated with oncogenesis, tumorigenesis, cancer cell vitality and proliferation, metastasis, immune evasion and drug-resistance (49). In the context of TDG, CBP/p300 acetylates certain lysine residues found in the TDG polypeptide,

preventing the high-affinity binding of TDG to the DNA strand and selectively inhibiting TDG identification of G:T mispairs (18). Interestingly, mutational analysis revealed that acetyl-acceptor lysine residues have no direct involvement in TDG attachment to DNA, but instead induce conformational changes to TDG that alter its specificity for DNA (18). Additionally, sumoylation at the C-terminus of TDG inhibits TDG interaction with CBP (7), potentially preventing acetylation and its effects.

Protein Kinase C α (PKCα) has canonically been implicated in regulation of cell adhesion, cytoskeleton structure, and cell migration, as well as being shown to promote differentiation in an anti-proliferative manner for certain tissue types but induce proliferation in others (50). Cancer tissue predominantly loses expression of PKCα, although in some cases tumor overexpression of PKCα is observed, with the increase in PKCα linked to promotion of chemotherapy resistance, increased migration, metastatic activity, and context-dependent induction or inhibition of cell proliferation or differentiation for cancer cells (50). PKCα interacts with TDG by phosphorylating serine residues on the N-terminal domain proximal to acetyl-acceptor lysine residues in a manner mutually exclusive to N-terminal acetylation (18). Phosphorylation does not significantly impact the DNA-binding ability of TDG, rather preserving this function by preventing the inhibitory acetylation by CBP/p300 (18).

Class-III histone deacetylase Sirtuin1 (SIRT1) plays critical regulatory roles in several intracellular processes, including transcriptional regulation, autophagy, maintenance of genomic stability, senescence, apoptosis, aging, and cell proliferation (51). The role of SIRT1 in tumorigenesis is highly context dependent, with aberrant SIRT1 expression associated with promotion of tumor development in certain cancers (e.g. melanoma and non-melanoma skin cancer, prostate cancer, primary colon cancer) and tumor suppression in others (e.g. breast cancer and hepatocellular carcinoma (HCC)) (51). SIRT1 was observed to interact with TDG at residues 67-110, catalyzing acetyl group removal from acetylated TDG, reversing functional effects associated with acetylation, and promotion of TDG glycosylase activity (16). Additionally, mouse embryonic fibroblast cells under TDG depletion observed increased TDG expression and acetylation (16). Taken together, these results indicate a role of SIRT1 in regulating the role of TDG in DNA repair, gene expression, and cytotoxicity due to genomic presence of 5flU (16).

Interestingly, SIRT1 overexpression is often observed in ER-positive breast cancer, where SIRT1 and ER interact to promote atypical intracellular signalling and abnormal transcriptional patterns which enhance tumor development (52).

Transcription Factor 4 (TCF4) is a loop-helix-loop structured transcription factor critical to the expression of several key genes, with aberrancies in TCF4 expression associated with several neurological and psychological disorders including Pitt-Hopkins Syndrome (53). Using coimmunoprecipitation assay, TDG

was found to interact with TCF4 to promote nuclear Wingless-related integration site (Wnt) signalling, a key process in the onset and progression of colorectal cancer (CRC), suggesting TDG may be a valuable biomarker for CRC (54).

Atypical TDG interaction with any of the above proteins may induce or inhibit tumorigenic effects, suggesting an indirect means by which TDG influences cancer growth.

Residues 328 to 410 span TDG's C-terminal domain, which binds Steroid Receptor Coactivator 1 (SRC1) and Thyroid Transcription Factor 1 (TTF1) (9, 10). Both SRC1 and TTF1 have been directly linked to tumorigenic behavior, suggesting - in a manner similar to that discussed above - an association between TDG molecular interactions and the onset or prevention of tumorigenesis. Canonically, SRC1 increases transcription of various genes by promoting formation of transcriptional complexes, enhancing chromatin accessibility, and binding to transcription factors as a transcriptional coactivator (55). Recently, SRC1 was identified as having an oncogenic role in the progression of tumor growth and metastasis for various types of cancer, especially those affecting the reproductive and urinary systems (e.g. breast cancer, prostate cancer, gastrointestinal cancer, etc.) (55). TDG has been demonstrated both *in vitro* and *in vivo* to interact with SRC1 at a motif found on TDG consisting of four tyrosine residues equidistant (separated by 3 amino acids) from one another (56). Though TDG-SRC1 interactions likely affect transcriptional activity to some extent, further research is necessary to clarify the exact genomic effect of this relationship.

Thyroid Transcription Factor 1 (TTF1) has been implicated in the development, maturation, and morphogenesis of various organs and tissues, with particular emphasis on the lung, forebrain, and thyroid (57). In lung cancer, TTF1 levels are most commonly increased, although research in the past years has demonstrated that TTF1 can exhibit either oncogenic or tumor suppressive activity in a context-dependent manner (57). TDG was identified as a highly influential and dose-dependent repressor of TTF1 transcriptional activity, with repression occurring in thyroid and non-thyroid cells at TTF1 responsive promoters (58). It has been suggested that TDG is a key repressor of premature TTF1 transcriptional activity during embryogenesis, as TDG has been observed to be highly expressed at gestational day 14.5 of mouse embryonic development (9).

Both the N-terminal and C-terminal domains of TDG interact with the $-CH_3$ domain of CBP/p300 (10), suggesting a level of versatility to TDG and CBP/p300 interaction, although current research does not appear to indicate any occurrence of acetylation activity at the C-terminus of TDG. Further research is needed to clarify the purpose of TDG C-terminal interaction with the $-CH_3$ domain of CBP/p300. Additionally, both the N-terminus and C-terminus of TDG interact with the N-terminus of Growth Arrest and DNA Damage-inducible α (GADD45α) (9). GADD45α has been identified to promote active DNA methylation

via interaction with TDG, increasing 5fC and 5caC excision (29). Supporting this relationship, knockout of both GADD45α and GADD45β results in hypermethylation at several positions in the genome, the majority of which are typically targets of TDG demethylation activity (59). Importantly, chromatin oligomerization by TDG is reversible via GADD45α activity, further connecting the TDG-GADD45α genomic relationship to transcriptional regulation (46). Disruption to this pathway may induce abnormal transcriptional activity associated with tumorigenesis.

The central domain of TDG is highly conserved, regulating key noncanonical molecular interactions such as binding of p53, Retinoic Acid Receptor α (RARα/RXRα), and Nuclear Receptor Coactivator 3 (NCOA3) (9, 10).

Highly influential and frequently studied tumor suppressor p53 plays significant anticancer roles in numerous cellular processes, including cell-cycle arrest, metabolism, immunity, senescence, apoptosis, and ferroptosis (60). To control TDG expression, p53 binds at the TDG promoter (9) and in turn, TDG potentiates p53 signalling (8). Additionally, Aranda et al. have identified in pluripotent cells that TDG and p53 co-bind specific regulatory elements to control gene expression, suggesting a role in cellular differentiation (46).

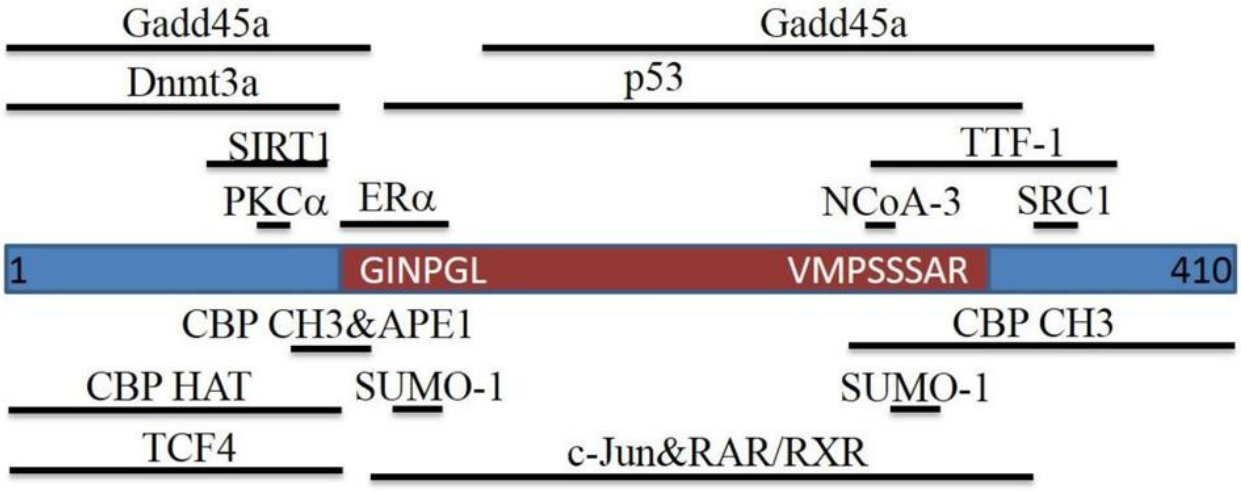

Figure 4. Structural Domains of TDG and the proteins to which they bind are depicted. Residues 1 to 111 comprise the N-terminal domain and residues 328 to 410 comprise the C-terminal domain. N- and C- terminal domains are represented in blue, whereas the central catalytic domain of TDG is represented in red. Various proteins are shown in black either above or below the region of TDG with which they interact (Figure adapted from Figure 3 in Xu et al. 2016: Reference 9 in this paper).

RARα and RXRα serve as binding points for Retinoic Acid (RA), a vitamin-A derived metabolite critical to cellular differentiation as well as embryonic development. The binding of RA to RARα or RXRα stimulates transcriptional activity and often results in downstream gene activation or inactivation essential to the aforementioned developmental

processes (61). The RA signalling pathway is often utilized for anti-cancer therapy for certain cancers such as acute promyelocytic leukemia, as well as breast and prostate cancer (61). TDG interacts with RARα to promote expression of RARα target genes. Additionally, TDG has been shown to form a ternary complex with both RARα and CBP which couples the previously considered distinct roles of TDG in transcriptional regulation and protection of the epigenome (62).

NCOA3, part of the Nuclear Receptor Coactivator (NCOA) family, serves as a coactivator for various transcription factors and nuclear receptors (NRs) to enhance transcriptional activity (63). Chiang et al. found that NCOA3-mediated promotion of NR transcriptional activity occurs in a manner dependent on NCOA3 interaction with TDG (64). Broadly, NCOAs have been implicated in cancer development and progression, with NCOA3 specifically linked to improved autophagy and found to oncogenically promote tumor expression (63). NCOA3 has been observed to be overexpressed in ovarian, esophageal, colorectal, and breast cancer, with transient NCOA3 depletion able to inhibit xenograft tumor growth and proliferation of nude mouse cancer cells (63).

3.2.2 *Oncogenic behavior*

TDG overexpression has been observed in many varying types of cancer, suggesting an oncogenic role. Abnormally high levels of TDG are a characteristic of HCC, the most predominant form of liver cancer, with TDG overexpression correlated to worsened prognosis for patients (65). Importantly, TDG demethylation activity has been demonstrated to regulate ABL Proto-Oncogene 1 (ABL1) expression (13). Thus, increased TDG demethylation at the ABL1 promoter may induce oncogenic behavior of ABL1 and as such allow for malignant progression of HCC via the Hippo signaling pathway (13). TDG is also expressed excessively within gliomas, the most common primary malignant tumors in the central nervous system, as well as being associated with poor prognosis (66). Remarkably, it has been noted both *in vitro* and *in vivo* that TDG depletion in glial cells as well as HCC cells inhibits the phenotypic malignance of the tumor (65, 66), implicating TDG as a potential target for glioma and HCC intervention therapies. For human CRC patients, TDG expression has been noted as significantly higher in tumor tissue compared to surrounding noncancerous tissue (9), with TDG knockdown in various CRC cell lines via short hairpin RNA (shRNA) introduction shown to effectively suppress tumor growth (54). Moreover, xenograft models demonstrate that TDG depletion inhibits melanoma tumorigenesis, furthering the notion that TDG plays a critical role in the onset and progression of cancerous growth (67). In melanoma cell lines, it has been shown that TDG depletion results in cell cycle arrest, senescence, or cell death (67). However, it is important to note that noncancerous melanocytes are only minimally affected by TDG depletion and adult mice under transient TDG knockout remain healthy (67), suggesting that TDG could serve as a potential target for melanoma intervention therapy. One study noted additionally that TDG levels were abnormally high for adrenocortical tumors expressing mutant (p.R337H) p53, with

the mutated p53 serving as a defining characteristic of cancer predisposition disorder (Li-Fraumeni Syndrome) variant Li-Fraumeni-like Syndrome (68).

TDG activity has been implicated in the formation of single strand breaks (SSBs) in the DNA double helix, leading to endogenous damage that could potentiate tumorigenesis. The TET-mediated active demethylation and BER pathways have been suggested to generate SSBs *in vivo*, particularly when rendered ineffective due to genomic changes associated with cancer, within chromatin regions either lacking methylation or under hydroxymethylation (69, 70). Unrepaired SSBs may stall or block activity of RNA polymerase, contributing to the formation of RNA or DNA hybrids, characterized via a displaced single-stranded loop, called R-loops at the site of transcription (71). Should they collide with a replication fork, R-loops may contribute to certain cytotoxic or clonogenic effects, potentially destabilizing the genome to initiate tumorigenesis (71). Intriguingly, although SSB generation has been associated with tumorigenic genome instability via several pathways, cellular diseases rendering SSB repair pathways ineffective have not to date been associated with an increased risk of cancer (71).

Aberrant TDG expression inhibited repair of DNA lesions induced through UV irradiation, often leading to cell death (72). However, it was found that initiating TDG mutagenesis to prevent BER-related functionality reversed the inhibition (72), potentially connecting TDG excision-related endogenous damage to functional defects with respect to UV irradiation.

Importantly, it has been reported that although SSBs may result from TDG-mediated nucleotide excision, double strand breaks (DSBs) are avoided due to the sequential manner in which TDG-mediated excision occurs for symmetrically methylated CpG nucleobase pairs (73). However, accumulation of TDG polymorph G199S, comprising ~ 10% of the global population, has been demonstrated to result in DSBs as well as cellular transformation, potentially due to the relatively tighter binding of this TDG variant to its substrates (9). The sequential process of active DNA demethylation may augment the potential for mutation originating from CpG dinucleotides (73).

Recently, TDG has been implicated in the transcriptional regulation of ER, a ligand-dependent member of the nuclear receptor superfamily to which TDG binds and functions as coactivator (8). In the presence of β-estradiol (E2), ER binds at gene-specific enhancer regions to promote transcription of certain noncoding enhancer RNAs (eRNAs), which in turn stimulates transcription of various surrounding genes (74). ER binding, regulated by E2, occurs at ~ half of all genomic TDG binding sites, and sites occupied by ER and TDG have been observed to actively promote eRNA transcription as well as mediate the epigenetic movement of DNA enhancer and promoter regions to the vicinity of target genes to promote gene expression in a TDG-dependent manner (8). Dysregulation of ER activity could thus result in the overexpression

of certain oncogenes, promoting tumorigenesis. TDG knockout was found to inhibit eRNA and ER target gene E2-mediated transcription (74), preventing gene expression. ER is overexpressed in many breast cancers, with its signaling pathway being a major target of endocrine-based intervention treatments (74).

TDG may also be involved in dedifferentiation of somatic cells to Cancer Stem Cells (CSCs) due to its role in Wnt signaling. CSCs, a subset of cancer cells with a comparatively increased potential to initiate tumor development versus other tumor cells (2), have been linked to tumorigenesis, metastasis, and the maintenance of tumor heterogeneity, and research relating to the development and importance of CSCs in the cancerous genome is emerging in the continuous search for new angles from which cancer can be combated (75). Contributing to tumorigenic heterogeneity, CSCs are capable of self-renewal and development into nonCSC offspring, with certain CSCs able to generate rapidly-proliferating tumors in addition to tumors consisting of differentiated cells (2). Wnt signaling has been associated with several cancer-relevant cellular processes, including tumorigenesis, cell fate determination, and establishment of stem cell pluripotency (76). TDG, in cooperation with CBP, has been identified as a transcriptional coactivator of the β-catenin/Ternary Complex Factor (TCF), and was ultimately observed to potentiate Wnt signaling as well as expression of Wnt target genes (76). Dedifferentiation, the process by which previously differentiated cells regress to a state of increased pluripotency and often regain the ability for self-renewal, has been strongly linked to CSC formation (75). Wnt signaling was associated with dedifferentiation, stem cell-like properties, and tumorigenic behavior of intestinal villus cells; breast cancer bone metastases dedifferentiation to generate CSCs; and intestinal epithelium dedifferentiation to initiate development and propagation of colon cancer (75). The stimulatory role of TDG in Wnt signaling suggests an indirect role in the establishment of CSCs, a relatively novel avenue by which TDG promotes tumorigenesis.

3.2.3 *Tumor suppressive behavior*

TDG activity *in vitro* is critical for the expression of various tumor suppressor genes, including Hypermethylated in Cancer 1 (HIC1), p15ink4b, Retinoic Acid Receptor β (RARβ), Small Heterodimer Partner (SHP), and p53 (8).

HIC1 expression occurs in a TDG dependent manner, with transient TDG depletion resulting in decreased HIC1 levels *in vivo*, likely due to observed hypermethylation at HIC1 promoter associated with gene silencing (77). Typically, active DNA demethylation occurs at the HIC1 promoter in the presence of RA (77), indicating a mechanism by which TDG mediates RA-dependent prevention of cancer growth.

Cyclin Dependent Kinase (CDK) inhibitor p15ink4b stimulates cell cycle arrest by disrupting cyclin-CDK binding activity, thus suppressing cell proliferation to prevent tumor growth (78). P15ink4b is one of the most frequently inactivated genes in tumorigenesis, with inactivation attributed in both leukemia and lymphoma to either deletion or hypermethylation of CpG islands in the

p15ink4b promoter (78). CpG hypermethylation suggests disruption to the TET-TDG active DNA methylation pathway, potentially due to changes in TDG expression. Moreover, Thillainadesan et al. observed that Transcription Growth Factor β (TGFβ) promotes active DNA demethylation at the p15ink4b promoter via the recruitment of TDG, with TDG depletion resulting in an absence of demethylation activity at the p15ink4b promoter (79).

RARβ has been implicated in several antitumorigenic cellular processes including apoptosis, differentiation, gene expression, and immune regulation, dysregulation of which may contribute to the onset of tumorigenesis (80). Similar to the context of HIC1 and p15ink4b expression, hypermethylation of CpG islands at the RARβ promoter is associated with gene silencing and observed within numerous epithelial carcinomas, including cervical cancer, suggestive of a role in the development and progression of cancer (80).

The relationship between TDG and tumor suppressor p53 is complex, with TDG increasing effects of p53 signaling (8). Subsequently, p53 mediates transcription and regulatory activity of TDG via binding to its promoter region, as well as recruiting excess TDG to the Wild-type p53-Activated Fragment 1 (p21WAF1) promoter to stimulate transcription of associated genes (9). P21WAF1 is considered a universal inhibitor of the cell cycle due to its role in prevention of cyclin-CDK complex formation, suggesting an indirect role of TDG in suppression of tumor development (81). Additionally, TDG is underexpressed in certain cancers, indicative of a role in tumor suppression. For example, TDG mRNA levels have been observed to be abnormally low in several myeloma cell lines as well as in pancreatic adenocarcinoma (9).

Conditional TDG knockout disrupts glucose homeostasis in adult mice and leads to the accumulation of Bile Acid (BA) in the liver and serum of male mice, rendering the organism to a prediabetic state as well as leading to HCC onset for primarily male mice (82, 83). Under the depletion of TDG, the function of the Farnesoid X Receptor (FXR) and Schönlein-Henoch purpura (SHP) regulatory cascade became dysregulated (82), and aberrant DNA methylation patterns were observed in the mice livers (83). FXR and SHP act as tumor suppressors of HCC, modulating glucose and BA levels to maintain cellular homeostasis and prevent the development of HCC. Thus, the role of TDG in FXR and SHP regulation implies indirect tumor suppressive activity in the context of HCC (82).

Interestingly, the influence of a sex bias has been indicated for certain other tumor suppressive roles of TDG. In response to TDG inactivation within the small intestinal and colonic epithelium, Antigen Presenting Cell (APC)-mutant mice underwent a ~ two-fold increase in small intestinal adenoma development, with the majority of mice for which this increase was significant being female (84). Similarly, the lowest quartile of TDG and APC expression identified in the human ColoRectal Cancer in The Cancer Genome Atlas (CRC TCGA) database is predominantly comprised of female patients

(84). These phenomena taken together suggest a sex-dependent function of TDG in the onset or inhibition of tumorigenesis.

The presence of TDG inhibits the spread of human Colon Cancer (CC). TDG was observed both *in vitro* and *in vivo* to prevent migration and invasion of human CC cells, via activities such as interference with siDNMT3A as well as stimulating through direct binding, the ubiquitination and subsequent degradation of DNMT3A (85). Additionally, decreased TDG levels due to a heterozygous missense mutation in the TDG-encoding gene has been identified in human rectal cancer (8). TDG underexpression can also be associated with late-onset hepatoblastoma development (8) (Table 1).

Table 1. Brief summary of TDG oncogenic or tumor suppressive functioning in specific cancer types. Note that further general associations between TDG and other types of cancer exist, due to the role of TDG in transcriptional regulation of various oncogenes and tumor suppressors, as well as the mutagenic potential of dysregulation of TDG-mediated excision in BER.

Cancer Type	Role of TDG	Reference
HCC	Oncogene: TDG overexpression is associated with worsened prognosis; TDG demethylation regulates ABL1 expression.	(13, 65)
HCC	Tumor Suppressor: TDG depletion disrupts glucose and BA homeostasis.	(82, 83)
Glioma	Oncogene: TDG overexpression observed, associated with worsened prognosis.	(65, 66)
CRC	Oncogene: TDG overexpression observed in tumor tissue.	(8, 9)
Melanoma	Oncogene: TDG depletion inhibits tumorigenic processes; results in cell cycle arrest, senescence, or cell death.	(67)
Adrenocortical Tumor	Oncogene: TDG overexpression observed in tumors expressing mutant (p.R337H) p53.	(68)
Breast Cancer	Oncogene: TDG acts as coactivator in ER signalling.	(8, 74)
Myeloma	Tumor Suppressor: TDG mRNA levels abnormally low in tumor tissue.	(9)
Pancreatic Adenocarcinoma	Tumor Suppressor: TDG mRNA levels abnormally low in tumor tissue.	(9)
Small Intestinal Adenoma	Tumor Suppressor: TDG inactivation resulted in increased small intestinal adenoma development for (majority female) APC-mutant mice.	(84)
Colon Cancer	Tumor Suppressor: TDG presence inhibits migration and invasion of human CC cells.	(85)
Rectal Cancer	Tumor Suppressor: TDG underexpression due to mutation in TDG-encoding gene identified in tumor tissue.	(8)
Hepatoblastoma	Tumor Suppressor: TDG underexpression associated with late-onset hepatoblastoma development.	(8)
Esophageal Cancer	Oncogene: TDG demethylation activity at the TAZ promoter leads to TAZ overexpression and contributes to increased cellular motility, proliferation, and radioresistance.	(87)

3.2.4 *DNA methylation and tumor development*

In the context of tumorigenesis, DNA hypomethylation is generally associated with the overexpression of oncogenes to allow for uncontrolled cell proliferation whereas DNA hypermethylation is typically associated with silencing of key tumor suppressors (1, 12, 35). Given the critical role of TDG in active DNA demethylation, alterations to TDG expression such as those described in Table 1 may contribute to the aberrant methylation patterns observed in the cancer genome, promoting or accelerating tumor development.

Several types of cancer demonstrate global DNA hypomethylation coupled with localized DNA hypermethylation during the transformation to malignancy (86). In Endometrial Cancer (EC), for example, over 50 instances of hypermethylation at tumor suppressor genes as well as several instances of hypomethylation at oncogene promoters have been recorded (86). Additionally, increased levels of 5mC associated with hypermethylation trends may increase the potential for spontaneous 5mC deamination to thymine, generating DNA lesions which may further decrease tumor suppressor functionality and induce cell proliferation. 5mC deamination to T is proposed to be the origin of the most common cancerous signature, cancer mutational signature 1 (3), demonstrating the genomic importance of active DNA demethylation via the TET-TDG pathway as well as effective DNA repair mechanisms.

3.2.5 *TDG-based intervention strategies*

The persistence of drug-resistance as a challenge to the efficacy of cancer treatment programs necessitates the development of novel therapeutic strategies based on various intracellular targets. Several studies note that the role of TDG in BER, DNA demethylation, and transcriptional regulation of various key proteins indicates its potential as a valuable target in cancer therapy. Particularly due to the interactions of TDG with several key tumor suppressors and oncogenes, controlling the nuclear expression of TDG could significantly affect the onset and progression of tumorigenesis.

One study proposes utilizing the tumor suppressive ability of TDG to prevent the development and spread of human CC cells via its function along the DNMT3A-TIMP2 axis to treat human CRC (85). Another study focusing on melanoma found that the introduction of certain TDG inhibitors reduced melanoma cell line viability and clonogenic capacity (67). An observed increase in 5caC indicated successful on-target activity of the inhibitors, with untransformed melanocytes undergoing little to no harmful impact, demonstrating the potential for targeted TDG inhibition as a means to treat melanoma in humans (67). Furthermore, TDG has been identified to sensitize MCF7 breast cancer cell lines to tamoxifen-mediated cytostasis, inhibiting tumor growth in response to tamoxifen-based hormone therapy (74). This, coupled with the regulatory role of TDG in MCF7 interaction with estrogenic and anti-estrogenic compounds and transcriptional regulation at certain MCF7 enhancers indicates the capacity of TDG-based intervention to alleviate MCF7 breast cancer (74). However, TDG has also been observed to increase migration and invasion of cancerous MCF7

cells (74), which must be considered within any potential TDG-based MCF7 breast cancer intervention strategies.

In esophageal cancer, overexpression of Transcriptional Coactivator with PDZ Binding Domain (TAZ) was associated *in vitro* and *in vivo* with increased cellular motility, proliferation, and radioresistance with the TAZ promoter observed to be hypomethylated due to TDG activity in the cancer genome (87). Moreover, increased TAZ expression consequently increased expression of genes associated with Nonhomologous End Joining (NHEJ), key players in the repair of irradiation-induced DNA damage, demonstrating a mechanism by which TAZ contributes to radioresistance (87). Resistance to radiation therapy contributes significantly to esophageal cancer relapse or metastasis (87). Thus, inhibition of tumor-enhancing activity at the TDG/TAZ/NHEJ axis could serve as a therapeutic method to combat esophageal cancer. It has also been suggested, due to the observed ability of cellular myelocytomatosis oncogene (c-Myc) to activate TDG-mediated demethylation of the Sterol Regulatory Element-Binding Protein 1 (SREBP1) via the increased presence of insulin and that of AMP-Activated Protein Kinase to inhibit TDG methylation activity via the decreased presence of metformin, that TDG may be an effective target for intervention against cancers associated with Type 2 Diabetes Mellitus (88).

Despite the potential benefits of TDG-based cancer intervention, several studies warn of potential damaging effects and challenges associated with the development of therapeutic strategies targeting TDG. One paper found that TET-mediated active DNA demethylation resulted in TDG-dependent endogenous DNA damage or even neuron death via disruption of the single-nucleotide gap-filling process at SSBs targeted at neuronal enhancers, which may have led to neurotoxicity post-anticancer treatment (89). Additionally, the wide variety of TDG functions in numerous signalling pathways may result in unexpected and potentially damaging concomitant genomic effects due to an increase or decrease in TDG expression. Likely, promoting or inhibiting only certain functions of TDG will be impossible without also impacting other unrelated TDG functions and associated cellular processes.

4. Perspectives

The destructive impact of cancer to human health coupled with the problematic frequency of drug-resistance in tumor tissue elucidates the importance of developing novel intervention strategies to combat the disease. Presently, TDG activity has been identified as imperative to the BER and active DNA methylation pathways, revealing the dual role of TDG in maintaining both genetic and epigenetic nuclear integrity. In addition, through transcriptional regulation and post-translational interaction with protein products, TDG mediates the activity of various key enzymes and transcription factors to determine cellular function. Alterations in TDG expression due to promotion or inhibition at the TDG promoter or chemical changes to TDG structure could consequently lead to dysregulation of TDG-dependent pathways, possibly altering function of key tumor suppressors or oncogenes,

demonstrating the potential of TDG as a target to prevent tumor development and proliferation for numerous cancers. Although several researchers propose various methods by which TDG can be targeted for cancer treatment, no TDG-based treatment has yet been developed or clinically tested in humans.

Dysregulation of TDG-dependent maintenance of genomic integrity can contribute both directly and indirectly to the development of the cancer microenvironment. One relatively unexplored relationship is that between the preventional role of TDG in oxidative stress-induced mutagenesis and the establishment of the cancer genomic landscape. Oxidized adenine and guanine derivatives 8-oxoA and 8-oxoG have been identified at abnormally high levels in several mammalian tumor tissues, including stomach, larynx, ovary, brain, and lung tissue (90), thus suggesting - due to BER being the major mechanism of oxoA excision - a dysregulation of TDG-mediated BER in cancer tissue. Activation of the synthetic Harvey Rat Sarcoma (HRAS) proto-oncogene, contributing to tumorigenesis, occurs as a result of 8-oxoG- or 8-oxoA-induced mutation (90). OxoA additionally has a high potential for genotoxicity due to its role as an origin of mutagenesis, as the significantly lower redox potential of 8-oxoA compared to adenine can result in further oxidation of the 8-oxoA nucleobase (90).

Interestingly, TDG-mediated excision of 5flU from DNA causes cell cycle delay or even arrest at S phase, as well as causing persistent stand breaks in DNA and stimulating a DNA damage response (17). Thus, cytotoxicity due to 5flU increases along with TDG expression levels, enhancing efficacy of 5flU-based cancer therapies (17). TDG expression levels may as such be an important determinant of cellular response to 5flU and serve as a predictor of the effect 5flU-based intervention has in the genome (17).

Comprising 6.2% of the global population, TDG variant D239Y has been shown to increase cellular sensitivity to DNA damage as well as playing a greater role in the DNA damage response (9). The role of TDG polymorphism, specifically in terms of how specific variants such as D239Y may impact antitumorigenic processes or potentially the efficacy of therapeutic intervention that damages tumor DNA may be deserving of further research.

The role of TDG in establishing and altering the cellular epigenome, most prominently through active DNA demethylation but also through the induction of chromatin oligomerization, warrants further investigation to elucidate the significance of this role in tumorigenesis. With respect to possible changes in DNA damage tolerance and transcriptional activity at associated genes, the role of chromatin condensation and the regulatory role of TDG in this process could influence the establishment of the cancerous genome. Although the TET-TDG active DNA demethylation pathway is currently the most effectively evidenced and widely accepted model of 5fC and 5caC removal, alternative pathways involving direct deformylation and decarboxylation of the respective bases have been proposed (91). A method of

demethylation involving direct removal of the formyl or carboxyl group from cytosine eliminates the potential for harmful single or double strand DNA breaks which can result from base excision (91), lessening the possibility of DNA damage that could result in dysregulation of critical anti-cancer pathways. Elucidating the true mechanism by which DNA methylation and demethylation occurs is critical to understand the means by which tumorigenesis initiates and progresses, and from there how it can be circumvented.

The observed sex-dependent bias in TDG activity deserves further examination, particularly in relation to the role of TDG in sex hormone, such as ER or androgen, signaling. Understanding the nature of this phenomenon may reveal valuable insight into both the intracellular effects of TDG regulatory activity and the context-based efficacy of TDG as a target of cancer therapy. Moreover, a more comprehensive understanding of the indirect influence of TDG-dependent molecular interactions and transcriptional regulation on cellular function is critical to predicting possible concomitant effects associated with TDG promotion or knockout in cancer progression or treatment. The multifaceted nature of TDG function and its moonlighting implies that alterations to TDG expression may result in harmful genomic consequences, which should be considered before developing any therapeutic strategy that utilizes TDG as a target. Potentially, harnessing the functional effect of specific chemical modifications (i.e. acetylation, phosphorylation, or sumoylation) or the truncation of certain TDG residues could be a means to reversibly alter TDG activity without holistic change to endogenous TDG levels.

The connection between TDG function and cellular differentiation is deserving of further exploration, particularly given the role of TDG in Wnt signalling. TDG accumulates during the G1 phase of the cell cycle (46), suggesting a function of TDG in cell-fate determination and exit from the cell cycle. Interestingly, although TDG is expressed in G1 and G2/M phases, existing TDG is degraded via ubiquitination at the G1/S boundary causing its cellular absence during the S phase of the cell cycle (17). This observation may suggest the potential for TDG expression to cause endogenous damage during DNA replication processes, a concept warranting consideration due to its implications for other effects of TDG functionality. RA-dependent differentiation fails to occur in the absence of TDG (8), likely due to the role of TDG in RA signaling. Importantly, it has been proposed that the RA signaling pathway would be a highly effective target of drug-based therapy to reduce and eradicate CSCs (92). TDG interaction with CBP/p300 was also established as crucial to euchromatin formation and synaptic signaling during cell fate determination (9). Additionally, the increased levels of 5fC at active enhancer and intragenic regions (36) suggests a link between TET-TDG active DNA demethylation and pluripotency. Several studies demonstrated in various contexts that transient TDG depletion resulted in a failure of cells to undergo differentiation processes, but the mechanisms behind this phenomenon remain somewhat unclear. Taken together, multiple avenues by which TDG expression influences differentiation processes

are presented, the understanding of which grows increasingly important with the emerging focus on CSCs' and their role in tumor growth and metastasis. Remarkably, somatic and pluripotent cells under transient TDG depletion demonstrate normal mutational rates (46), which appears to contradict the importance of TDG BER function suggested by other studies. However, more research is needed to fully understand the significance of this observation.

4.1 Potential of chimeric technology to correct dysregulated TDG expression

Dysregulated TDG expression induces several tumorigenic effects, with TDG itself functioning as either an oncogene or tumor suppressor in a context-dependent manner. As a general trend observed across currently published studies, TDG overexpression has been associated with oncogenic effects, and TDG underexpression drives cancer progression as well, likely due to reduced rates of critical TDG-dependent tumor suppressive processes. Therapeutic intervention involving Proteolysis-Targeting Chimeric (PROTAC) technologies may act to normalize the concentration of TDG in cancerous tissue to that of surrounding healthy tissue in order to counteract the contributory role of dysregulated TDG expression in tumorigenesis.

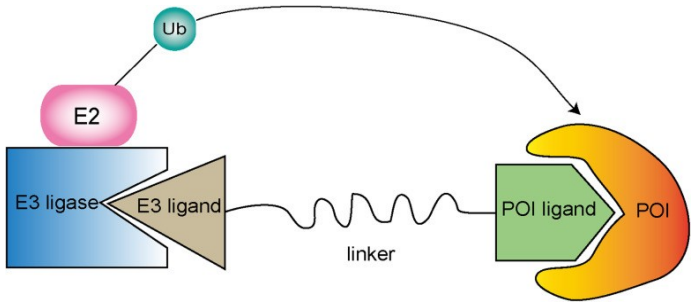

Figure 5. The molecular structure of PROTAC is depicted. A chemical linker attaches a POI ligand to a E3UL ligand, selectively binding the POI and E3UL respectively to form a POI-PROTAC-E3UL ternary complex, reducing the physical distance between E3UL and the POI to facilitate POI polyubiquitination. (Figure adapted from Figure 5 in Yang et al. 2021: Reference 94 in this paper.)

PROTAC takes advantage of the intracellular Ubiquitin-Proteasome System (UPS) to selectively facilitate proteolysis of a certain protein of interest (POI) (93). Comprised of a POI ligand connected via chemical linker to an E3 Ubiquitin Ligase (E3UL) ligand, PROTAC mediates the recruitment of E3UL into proximity of the POI, promoting polyubiquitination of the POI by E3UL via the formation of a POI-PROTAC-E3UL ternary complex (Figure 5) (93). E3UL recruits an E2 Ubiquitin Ligase (E2UL) carrying activated ubiquitin and catalyzes the selective transfer of ubiquitin from E2UL to form an isopeptide bond at lysine, serine, threonine, or cysteine residues in the POI (94). Each ubiquitin molecule contains a specific lysine residue, Lys 48, at which another ubiquitin may attach to

facilitate polyubiquitination (95). Polyubiquitination marks the POI for recognition and subsequent degradation by the 26S proteasome complex through the UPS (93). Importantly, PROTAC functions catalytically in a sub-stoichiometric manner, meaning that each individual PROTAC may induce proteolysis of several POI molecules (96), enabling high rates of degradation even at low concentrations of the drug.

By developing a PROTAC molecule targeting TDG as its POI, overexpression of TDG may be corrected, preventing oncogenic effects as seen in certain cancer types including HCC, glioma, and CRC. However, introduction of TDG-targeting PROTAC to cells expressing a normal or abnormally low amount of TDG may result in cellular toxicity. Similarly, PROTAC activity in noncancerous cells may lead to cytotoxic effects. Liang et al. proposed an enzymatically-activated Pro-PROTAC molecule that mediates proteolysis in a cell-specific manner, where an enzyme-responsive chemical capping molecule on the E3UL ligand prevented recruitment of E3UL by PROTAC, effectively disrupting any proteolytic effect except in the presence of the specific enzyme which may "uncap" the E3UL ligand (97). In a proof-of-concept study for the Pro-PROTAC mechanism, trimethyl-locked quinone bound as a capping molecule to the E3UL ligand site, preventing E3UL recruitment and consequently blocking PROTAC-induced proteolytic activity (97). Reduction of the trimethyl-locked quinone by NAD(P)H Quinone Dehydrogenase 1 (NQO1), which is commonly overexpressed in tumor tissue, could result in its dissociation from the E3UL ligand. Thus, PROTAC-induced degradation of the POI occurs predominantly in cancer cells as opposed to noncancerous cells (97).

Presence of a "capping molecule" could potentially be utilized to prevent PROTAC-induced degradation of TDG in tumor cells expressing normal or low TDG levels, reducing the likelihood for cytotoxic effects. To achieve this effect, a TDG-responsive capping molecule that binds to the E3UL ligand component of PROTAC could prevent recruitment of E3UL (Figure 6). The conformational structure of the capping molecule could be dependent on a chemical bond similar to that which is cleaved by TDG during the BER process, creating a mechanism whereby a higher concentration of TDG could proportionally induce transient dissociation of the capping molecule from the E3UL ligand and thus activate Pro-PROTAC to degrade the excess TDG. A TDG-responsive capping molecule would allow for the activation of PROTAC to be reversible as endogenous TDG levels are reduced. In the presence of a lower concentration of TDG, the TDG-responsive capping molecule would remain associated with Pro-PROTAC to prevent degradation of TDG.

While appealing in a theoretical sense, development of TDG-responsive Pro-PROTAC technology to reduce TDG expression faces several challenges. Development would require the identification of a suitable ligand to bind TDG as well as a capping molecule that both prevents E3UL recruitment by PROTAC and is responsive to cellular concentrations of TDG. Additionally, identification of a suitable linker between E3UL and TDG ligand that facilitates

optimal spatial positioning for PROTAC-mediated ubiquitination of TDG may prove challenging (98). Finally, drug formulation studies may face challenges related to drug solubility and cell penetration due to PROTAC's relatively high molecular weight, challenges previously overcome during development of other PROTAC drugs (93). The molecular weight of PROTAC violates Lipinski's rule of five (ROF), which raises significant concerns regarding oral therapeutic efficacy. The addition of a chemical capping molecule would worsen the extent to which the PROTAC structure transgresses boundaries imposed by the ROF. Importantly, the significant intracellular reduction in POI levels mediated by PROTAC is expected to result in a similar phenotype to established genetic modification technology such as small interfering RNA (siRNA), shRNA, or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) (96), which may not always be an ideal outcome given that abnormally low levels of TDG are also associated with the onset of tumorigenesis and would disrupt TDG-mediated processes critical to cellular function. Ideally, addition of a TDG-responsive capping molecule would mitigate excessive degradation activity of PROTAC to prevent potential cytotoxicity.

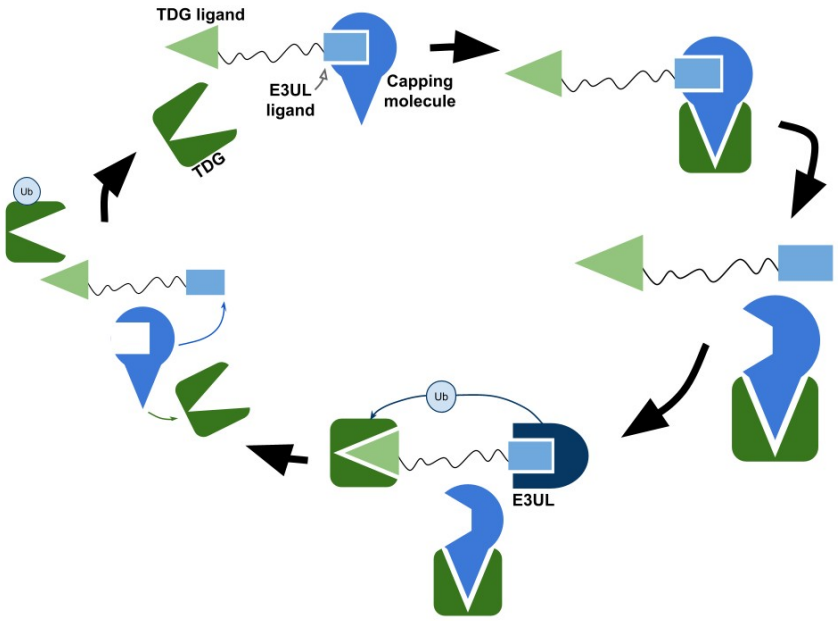

Figure 6. Binding of a TDG-responsive chemical capping molecule to the E3UL ligand may effectively regulate PROTAC activity to prevent cytotoxic effects. Excess TDG present in the cell could bind allosterically to the capping molecule, resulting in a reversible change to the conformational structure of the capping molecule that induces dissociation from PROTAC. Thus, E3UL may be recruited by PROTAC to ubiquitinate a distinct TDG. The release of TDG from the allosteric binding site allows the capping molecule to regain its active structure and bind once again to PROTAC, preventing further proteolytic activity.

An approach structurally similar to PROTAC that facilitates sumoylation rather than ubiquitination of TDG may aid in the development of drug-based therapeutic intervention targeting tumor tissue wherein TDG is underexpressed. A Sumoylation-Targeting Chimera (SUMTAC) facilitating conjugation of SUMO to TDG could increase enzymatic turnover rates of TDG in the BER and active DNA demethylation processes (Figure 7). The TDG ligand would preferentially bind DNA-bound TDG as opposed to free TDG, thus preventing potentially harmful alterations to free TDG binding activity. The catalytic efficiency of existent TDG in TDG-underexpressing cells could as such be maximized to produce phenotypes similar to those produced by typical amounts of TDG functioning at a normal rate.

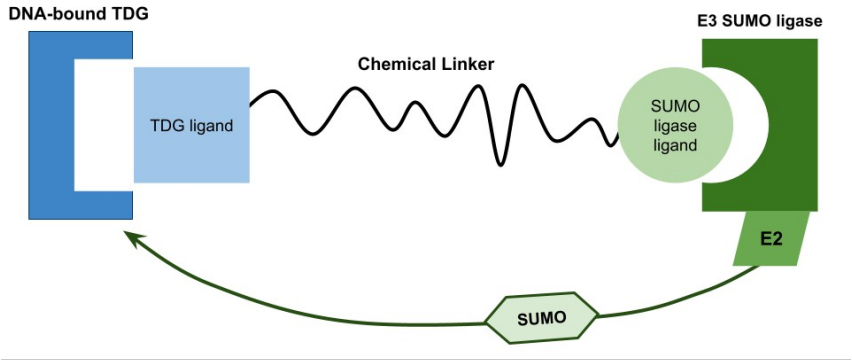

Figure 7. A SUMTAC molecule facilitating sumoylation of DNA-bound TDG is depicted. A chemical linker attaches a TDG ligand to an E3 SUMO ligase ligand, selectively binding DNA-bound TDG and E3 SUMO ligase respectively to form a TDG-SUMTAC-E3 SUMO ligase ternary complex, reducing the physical distance between E3 SUMO ligase and TDG to facilitate sumoylation of TDG.

It was demonstrated *in vitro* that dissociation of TDG from the substrate AB site is the rate-limiting step regulating TDG cellular activity (99), suggesting that a more rapid dissociation of DNA-bound TDG could increase overall cellular activity of TDG. At the initiation of physical contact with substrate DNA, TDG undergoes a significant change in conformational structure involving its flexible N-terminal domain, enabling it to bind tightly to DNA in a nonspecific manner, facilitating interaction with a wide variety of DNA mispair lesions but inhibiting dissociation of TDG from the resultant AB site, which in turn reduces enzymatic turnover (100). The alteration to TDG structure upon binding to DNA may aid in the identification of a suitable ligand, for example by ligand based screening technologies, to target SUMTAC activity preferentially to DNA-bound TDG. Sumoylation substantially reduces the binding affinity of TDG to DNA, likely via the stabilization of an α-helix (residues 317–329) incompatible with DNA, resulting in the rapid dissociation of DNA-bound TDG from the AB site, releasing TDG for subsequent reactions (99, 100, 101). Certain studies have found that the conformational shape changes undertaken

by TDG bound at an AB site of DNA stimulate SUMO conjugation (99), suggesting a mechanism whereby sumoylation is used as a regulatory mechanism to release TDG after the cleavage of its target nucleobase and coordinate the initial steps of BER.

Sumoylation of TDG occurs in a dynamic and easily reversible manner (99, 102), with the process of SUMO conjugation highly reminiscent of ubiquitination (100). E3 ligases, such as Protein Inhibitor of Activated STAT (PIAS) or RAN Binding Protein 2 (RANBP2), catalyze the transfer of an activated SUMO from an E2 conjugating enzyme, UBC9, to a lysine residue located on the target protein (100). TDG specifically interacts with and covalently binds to SUMO-1 or SUMO-2/3 at lysine residue 330 to promote its dissociation from an AB site (100). Deconjugation of SUMO is facilitated by several SUMO-specific proteases, notably Sentrin-Specific Protease (SENP) 1, which has been implicated as highly relevant to the regulation of TDG sumoylation levels (41). The reversible nature of sumoylation demonstrates that while conjugation of SUMO may transiently disrupt DNA-binding affinity of TDG, its active unmodified form can be easily restored via naturally occurring enzymes in the cell. The similarity between ubiquitination and sumoylation pathways, involving activating E1, conjugating E2, and ligating E3 enzymes, suggests the efficacy of a PROTAC-like structural model in a sumoylation context.

Increased rates of DNA-bound TDG sumoylation could enhance stimulatory effects of APE1 in regard to TDG turnover, improving catalytic efficiency to counteract effects of TDG underexpression in tumor tissues. APE1 has been associated with increased catalytic efficiency of non-sumoylated TDG, with one study finding that the presence of APE1 increases TDG turnover rates 42- and 26- fold for, respectively, G:T and G:U mispair lesions (101). Additionally, APE1 presence has been observed to significantly accelerate the release of sumoylated TDG from DNA, with the stimulatory effect of APE1 in regard to TDG turnover observed to be higher, due to a higher release rate of TDG from DNA, when SUMO is covalently bound to TDG compared to unmodified TDG (99). As a consequence of this phenomenon, sumoylation of TDG enables a relatively lower endogenous concentration of APE1 to produce the maximal stimulation of TDG turnover (101). For example, a 5 nm APE1 concentration had no significant effect on turnover of unmodified TDG, but successfully increased turnover of sumoylated TDG (101).

Importantly, although sumoylation of DNA-bound TDG during BER could increase turnover rates, the premature dissociation of TDG from an AB site before the arrival of APE1 to continue the process may leave the unstable AB DNA susceptible to SSBS or even DSBs, enzymatic degradation, and errors during replication or transcription (9, 100). Excessive drug-induced sumoylation of free TDG or insufficient rates of SUMO deconjugation by SENP1 or other relevant SUMO-specific proteases could lead to an abundance of sumoylated TDG in the nucleome. The lower affinity toward DNA as well as other functional attributes of

sumoylated TDG may reduce overall catalytic efficiency of free TDG or disrupt crucial TDG-mediated processes, resulting in cytotoxic effects.

To normalize TDG expression throughout the entirety of a tumor which may (1) overexpress TDG in certain tissues/organs but underexpress TDG in others or (2) demonstrate fluctuating or indeterminate TDG levels, a chimeric drug capable of selectively modifying its mechanism of action in response to variation in intracellular condition (i.e. varied levels of TDG expression) may prove effective. The drug must independently sample TDG concentration and facilitate interaction with the appropriate enzyme or enzymatic pathway to correct any abnormality, regardless of tissue or organ.

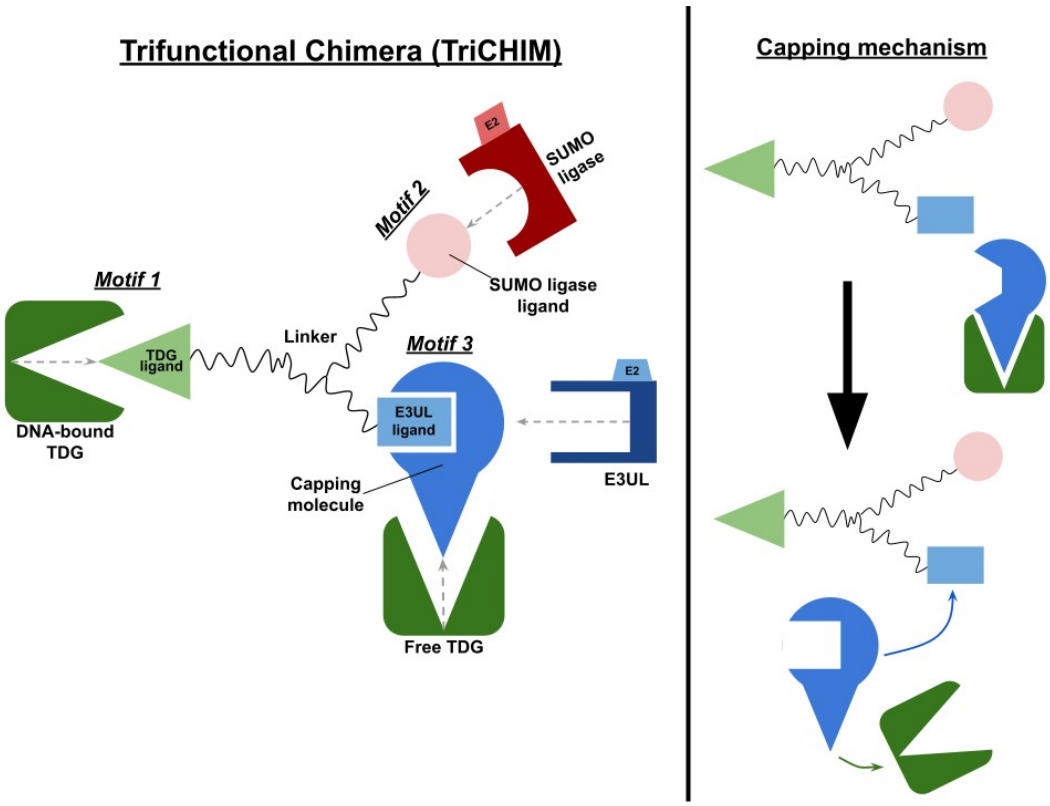

Figure 8. The structure of the proposed TriCHIM molecule and associated enzymes are depicted on the left. Structural motif 1, a TDG ligand, binds specifically to DNA-bound TDG. Motif 2, a SUMO ligase ligand, recruits SUMO ligase to facilitate sumoylation of DNA-bound TDG. Motif 3, a "capped" E3UL ligand, remains nonfunctional until free TDG interacts with the capping molecule. As depicted on the right, interaction with free TDG causes the capping molecule to dissociate from TriCHIM. At this point, motif 3 becomes active and TriCHIM may recruit E3UL to facilitate ubiquitination of target TDG. All three structural motifs are joined via a chemical linker.

A heterotrifunctional compound with the ability to induce either ubiquitination or sumoylation of TDG in a context-dependent manner may, in theory, satisfy these criteria. The trifunctional chimera (TriCHIM) would possess three structural motifs, joined together via chemical linker (Figure 8). Similar to the traditional PROTAC molecule, motif 1 would be a POI-specific target ligand, serving in this instance to engage DNA-bound TDG. Motif 2 attracts some enzyme(s) to upregulate or stimulate POI activity. Here, an E3 SUMO ligase ligand would be used to achieve the same effect, increased TDG turnover, as SUMTAC (described previously in this section). To induce degradation via the UPS system if and only if POI levels are abnormally high, a POI-responsive "capped" E3UL ligand, as described previously in the context of Pro-PROTAC, would serve as motif 3.

Under the condition wherein TDG is underexpressed, the E3UL ligand would remain capped. Thus, motif 3 would be rendered to an inactive state, where E3UL is not engaged and as such the POI is not degraded. Instead, DNA-bound TDG would interact with motif 2, facilitating sumoylation of TDG which in turn increases its turnover and mimics phenotypes produced by typical TDG expression levels. Under the condition wherein TDG is overexpressed, free TDG would interact with the "cap" on the E3UL ligand, catalyzing its reversible dissociation from the E3UL ligand and activating motif 3. Motif 1 would bind DNA-bound TDG, and motif 2 would still catalyze TDG sumoylation and thus dissociation from DNA. However, TDG would remain bound at motif 1, and could thus interact with motif 3, which recruits E3UL to facilitate conjugation of ubiquitin to TDG and induce proteolysis via the UPS system. As such, intracellular TDG concentration would be reduced. Given both that TriCHIM acts in a stoichiometry-independent manner and that "cap" dissociation from motif 3 is reversible, introduction of a low dose of TriCHIM may enable relatively long-term normalization of TDG levels.

However, practical development of TriCHIM technologies would likely prove challenging, necessitating meticulous effort to identify suitable ligands, capping molecules, and linker lengths. Incorporating a third structural motif implies a significantly higher complexity compared to traditional PROTAC, augmenting time and effort needed for design and testing processes. Additionally, higher molecular weight compared to traditional PROTAC would almost certainly violate the ROF, suggestive of significant difficulty in developing effective TriCHIM technology. Further research clarifying the existence and/or nature of crosstalk between modification by SUMO and ubiquitin would likely be necessary to ensure TriCHIM efficacy as described in this section.

5. Conclusion

Despite the continuous emergence of new research in the field of cancer, the disease continues to pose a severe threat to human health. Thus, enhancing our understanding of the mutagenic origin of tumorigenesis and identifying novel targets for therapeutic intervention strategies grows increasingly important. TDG has been demonstrated to be

crucial in defending the integrity of both the genome and the epigenome, most directly through its role in BER and active DNA demethylation, but also indirectly through its various interactions with key tumor suppressors, oncogenes, and proteins involved with genomic maintenance.

The behavior of TDG in tumorigenesis is somewhat enigmatic. Dysregulation of TDG-dependent pathways may contribute to the onset of tumorigenesis due to increased potential for mutation or abnormal transcriptional activity that allows for uncontrolled cell proliferation. TDG overexpression is observed in several cancers, suggestive of an oncogenic role, with TDG knockout inhibiting progression of tumorigenesis. However, TDG also has been identified to act as a tumor suppressor in a context-dependent manner. The role of TDG in safeguarding the methylation status of the genome also influences tumorigenesis, with hypermethylation generally being associated with silencing of key tumor suppressors and hypomethylation with the overexpression of oncogenes. Utilizing PROTAC or Pro-PROTAC to induce degradation of TDG may effectively reduce TDG expression in TDG-overexpressing cancers. SUMTAC, a proposed novel drug based on the PROTAC structural model that induces sumoylation of TDG, could increase TDG turnover rates to counteract effects of TDG underexpression. In addition, a proposed trifunctional chimera could theoretically be utilized to normalize TDG concentration throughout an entire tumor regardless of whether TDG is overexpressed or underexpressed.

Overall, the nuanced role of TDG in cancer development suggests that TDG may serve as a valuable target of intervention therapy for various types of cancer. However, understanding of the multifaceted intracellular role of TDG is essential to avoid potentially harmful concomitant endogenous changes associated with altered TDG expression due to TDG moonlighting in post-translational regulation of protein activity.

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