Review

Untousling the role of Tousled like kinase 1 in DNA damage repair

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Untousling the Role of Tousled like Kinase 1 in DNA Damage Repair

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Abstract: DNA damage repair lies at the core of all cells' survival strategy, including cancerous. Therefore, targeting such repair mechanisms forms the major goal of cancer therapeutics. The mechanism of DNA repair has been tousled with the discovery of multiple kinases. Recent studies on Tousled like Kinases have brought significant clarity on the effectors of these kinases which stands to regulate DSB repair. In addition to their well-established role in the DDR and cell cycle checkpoint mediation after DNA damage or inhibitors of replication, their suspected involvement in the actual DSB repair process has more recently been strengthened by the important finding that TLK1 phosphorylates RAD54 and regulates some of its activities and localization in the cell. Earlier findings of its regulation of RAD9 during checkpoint deactivation as well as defined steps during NHEJ ends processing were earlier hints of its important involvement broadly in DSB repair. All this has opened up new avenues to target cancer cells in combination therapy with genotoxins and TLK inhibitors.

Keywords: TLKs; DNA repair; HRR; NHEJ; replication; DNA damage; therapeutics

Introduction

Tousled like Kinases (TLKs) are Ser/Thr kinases which were discovered with potential functions in DNA replication and in DNA damage repair nexus in higher eukaryotes [1–3]. There are two homologs, TLK1 and TLK2 which shares 94% amino acid sequence identity in the kinase domain and an overall 84% identity. There is significant overlap between the substrates of TLK1 and TLK2 so far obtained from in vitro mass-spectrometry in different studies [4]. However, distinct biological roles of the paralogs are forthcoming, as not all their substrates are common. Their functional overall was addressed with viability studies with mouse embryos, which showed that TLK1 is important for later development stages whereas TLK2 is required for placental development. The most well-supported role of TLK1 is in DNA damage response and replication. TLK1 depletion has been shown to delay S-phase progression [5]. This is possible because TLK1 has been found to interact with human RAD9 during replication fork stalling. Phosphorylation of RAD9 (S328) by TLK1 has been shown to dissociate 9-1-1 complex thereby causing cytosolic localization of RAD9 and thus participate in the deactivation of the checkpoint after completion of DNA repair [6]. There has been increasing appreciation for structural domains in kinases that can act as scaffolding units to recruit substrates for their catalytic function. Interestingly TLK1 also possesses chaperone function that recruits RAD9 at the junction of dsDNA and ssDNA at DSB site [7]. Another downstream target of TLK1 is Asf1a/b which is phosphorylated at its C-terminus during S-phase and that leads to binding of H3-H4 tetramer that is assembled on newly replicated DNA. TLK1 can directly bind to chromatin and its interaction with chromatin has been also linked to replication stress, upon which, TLK1 binding to chromatin decreases [7,8]. TLK1B, a splice variant of TLK1 is expressed upon IR exposure and provides radio-resistance to cells. TLK1B overexpression has been also shown to induce UV resistance [9]. TLK1 and TLK1B share the conserved kinase domain and therefore, TLK1 and TLK1B have considerable substrate overlap. One such substrate is Asf1b which is phosphorylated by TLK1/B and has been shown to gain chromatin remodeling activity in cell extracts [9]. Similarly, TLK1 dependent phosphorylation of Asf1a leads to an increase in histone octamer recruitment at newly

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repaired DNA strands [8]. In another model organism *T. brucei*, TLK1 interacts with Asf1a and Asf1b to phosphorylate the histone chaperones which helps to maintain their activity [5].

Role of TLK1 in DSB repair

In vitro Tousled like Kinase 1 can bind directly with many DNA damage and DSB repair-related proteins like - NEK1, AKTIP, RAD54B, FANCM and others [10]. A more specific role of TLK1 in regulating DSB repair via RAD54 has recently been discovered [11]. This is further addressed below, but RAD54 along with its partner RAD51 (the key recombinase of eukaryotes) performs much of the work in the HRR process, which has led to naming RAD54 the Swiss army knife of HRR [12].

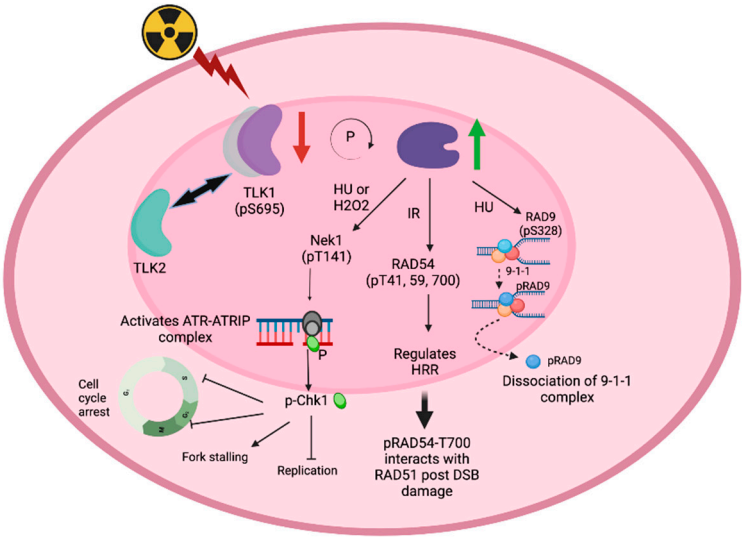

Figure 1. Mechanistic role of TLK1 in DDR. TLK1 (light purple) can heterodimerize with TLK2 (cyan) in certain cell types and normal kinase activity of TLK1 is dependent on homodimerization/oligomerization and/or heterodimerization. The N-terminal region of TLK1/TLK2 is essential for higher order arrangement. In cells, when DSB is induced with IR, there is a transient decrease in TLK1 activity (red arrow) which is restored within an hour (in HeLa cells). The inhibition of TLK1 is dependent on ATM-Chk2 phosphorylation at S695 (or equivalent S457 of TLK1B, spliced isoform). Activation of TLK1 (dark purple) is dependent on its autophosphorylation (shown by circular arrow). When TLK1 is active following DSB damage (green arrow), TLK1 phosphorylates multiple DDR substrates. TLK1 can phosphorylate RAD54 particularly at T41, T59 and T700 which has been investigated in the current study. During replication stress by HU or oxidative damage by H2O2, TLK1 can phosphorylate Nek1 at T141 which leads to ATR-ATRIP complex (grey spheres) activation that function upstream of Chk1 (green sphere). Chk1 is phosphorylated within nucleus and inhibits replication, leads to fork stalling and arrests cell cycle at G1/S, intra-S phase and G2/M checkpoints. During HU dependent stress, RAD9 (light blue) is phosphorylated by TLK1 at S328 in 9-1-1 complex at replication fork. Phosphorylated RAD9 (dark blue), dissociates from 9-1-1 complex and shuttles to cytoplasm after recovery of DNA damage. Figures were designed in BioRender.com.

Upon DSB induction by IR, TLK1 is transiently inhibited via a Chk1 dependent mechanism, but it is important to note that depletion of Chk1 followed by irradiation did not decrease the TLK1 activity in HeLa cells. Further, HeLa model cells lack functional p53 and therefore, the decrease in TLK1 activity post IR may be p53 independent [3]. Initially TLK1 was peptide mapped to be phosphorylated by Chk1 at Ser 695 that lies within the kinase domain [3] but later studies aligned Ser 743 as the site of phosphorylation that marks TLK1 inactivity [13,14]. The sub-cellular localization of full-length human TLK1 in HeLa cells is mostly nuclear with a diffused signal pattern [1]. Post DSB induction with Mitomycin C, we observed distinct TLK1 foci that partly colocalize with H2A.X foci 2hrs after DNA damage [11]. This suggests that TLK1 plays an important role in DSB repair as most

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probably it promotes convergence of a hub of factors that function downstream in repair pathways. RAD54 and RAD51 co-localizes to form foci with slightly delayed kinetics than those formed by TLK1 (approx. 4hrs post-induction) and RAD54-51 foci persists till 10hrs. RAD9 on the other hand has been found to form foci rapidly post DNA damage independent of RAD52 group foci localization and this has been assigned to the checkpoint related function of RAD9 [15] and potentially to a preferential involvement in NHEJ [16–19].

With different RAD proteins serving as TLK1 substrates, these observations suggest that TLK1 can interact and phosphorylate different RAD proteins and regulate DSB repair, via either HRR or NHEJ, in a concerted and perhaps sequential fashion.

Role of TLK1 in regulating HRR factors

A recent study shows that TLK1 can phosphorylate RAD54 and regulate different stages of HRR. TLK1 phosphorylates RAD54 at both the N-terminal and C-terminal domains [20]. While the N-terminal domain has been shown to be a regulatory domain of RAD54, the C-terminal domain is a critical domain for contacting the double-strand DNA template and therefore serves as functional domain of the protein. Phosphorylation at both T41 and T59 of RAD54 can serve as an interacting platform for several known proteins of HRR for e.g RAD51AP1, NUCKS1, CDK2 [21] Maranon, 2020 #221} [22]. Interestingly, phosphorylation at the C-terminal domain (T700) can alter the intra-protein ionic environment within the Zn-finger like motif thereby causing a major change of interaction with its partner protein RAD51. Depleting TLK1, as shown by our previous study and others, exhibits a delayed S-phase progression phenotype [23,24]. HRR is a major participant of replication fork reversal. As recent studies have shown that RAD54 restrains replication fork progression when cells are stressed [25], the phosphorylation of RAD54 by TLK1 could play a significant role in modulating the dynamics of fork progression vs regression (or even reversal) as a major player for enacting accuracy and processivity at sites where DNA lesions are detected. An earlier explanation for the slow S-phase progression was attributed to a defect in chromatin assembly during the replication process due to the established role of TLKs as regulators of Asf1. However, it was also noted that the phenotypes from depletion of TLKs vs those observed with depletion of Asf1 are quite different with respect to the state of chromatin assembly and cell cycle progression [24]

Role of TLK1 in eukaryotic recombination

TLK1 activity is important for Homologous recombination repair. Activation of TLK1 activity using Gallic acid has been shown to increase HRR activity in cells [26]. TLK1 depletion by siRNA or shRNA methods across different cell lines has been shown to decrease HRR efficiency in cells significantly [11,23]. In a complementary approach, inhibition of TLK1 with thioridazine, led to a much greater accumulation of γH2Ax foci due to unrepaired DSBs [27].

TLK1 interactome reveals that RIF1 is a possible in vivo target [4,28,29]. RIF1 acts at the decision-making junction of NHEJ vs HRR, downstream of 53BP1 [30,31]. It is known to turn on NHEJ while inhibiting 5'-end resections in HR. Asf1a/b (NTD, 1-154 a.a) has been found to be interacting with RIF1 (N-terminus, 967-1350 a.a) independent of its chaperone activity [32]. Although TLK1 interacts with both Asf1a/b and RIF1, it remains to be elucidated whether TLK1 can impinge on NHEJ vs HRR decision by regulating RIF1, and further if it is dependent on TLK1 kinase activity or its chaperone function. In mammalian cells, RIF1 binds to aberrant telomeres in an ATM-53BP1 dependent manner when telomeres are unprotected and recognized as sites of DNA damage [33]. TLK1 depletion has been shown to increase telomeric sister chromatid exchange, thus indicating a state of hyper-recombination [34]. This implies that TLK1 can regulate telomeric recombination as well, and possibly mediate chromosomes fusion during aberrant cancer hyper-recombination.

TLK1 has been found to interact with another human paralog of RAD54 i.e, RAD54B which in yeast (Rdh54) localizes to DNA damage foci in a RAD52 dependent manner [10,15]. In vitro TLK1 phosphorylates RAD54B at T73 (unpublished data). Interestingly, the localization of Rdh54 in kinetochores has been shown to be RAD52 independent. Since TLK1 has been shown to function in chromosome segregation in different organisms [23,35,36], it is speculated that TLK1 can regulate

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RAD54B functions in mitotic or meiotic events of chromosomes dynamics. TLK1 preferentially localizes to the nucleolus even without DNA damage induction in HeLa cells [20]. The nucleolus is the compartment that drives ribosomal biogenesis and therefore, it is speculated TLK1 may play a role in ribosome biogenesis or have a specific role in DSB repair at this site, known to be comprised of highly compacted chromatin and highly repetitive sequences that presents additional challenges [37].

Role of TLK1 in cancer

In different disease models like prostate cancer (PCa), glioblastoma (GBM), TLK1 depletion has been shown to elicit DNA damage response and therefore TLK1 forms a druggable target [38–40] (Figure 2). GWAS analyses revealed that TLK1 mutations are rare in cancer but rather its overexpression is frequently linked to poor prognosis [34,41]; this is particularly evident for patients with low Gleason scores (e.g, GS=6 - Ualcan.path.uab.edu/analysis page 2), which would otherwise be expected to fare better survival. Likewise, amplification of TLK2 has been reported as a frequent event in breast cancer [42], GBM [43], and neuroblastoma [44]. Our previous work shows that DNA damage activates the TLK1> NEK1> YAP1 axis, which either further elevates the apoptotic pathway in PCa [45] or can lead to compensatory adaptation to genotoxins [46].

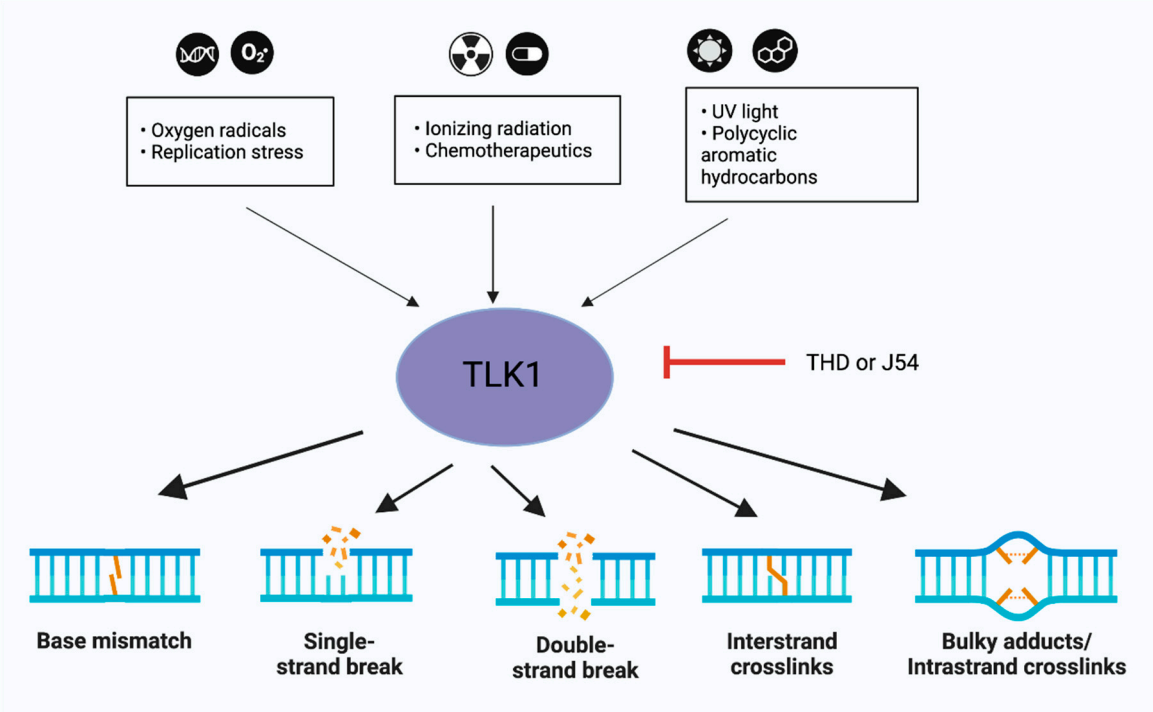

Figure 2. TLK1 at the core of DNA repair mechanisms. TLK1 forms a therapeutic target in multiple tissue specific cancer models. Inhibiting TLK1 can affect the base-excision repair, single-strand break (SSB) repair, double-strand break (DSB) repair or Inter/Intra-strand crosslink (ICL) repair in cells. The small black arrows indicate DNA damaging agents which activate TLK1 while the bold black arrows indicate that inhibiting TLK1 affects the specific DNA repair pathways. Figures were designed in BioRender.com.

Conclusion: Targeting TLK1 for cancer treatment

After the seminal discoveries that TLK1 has important modulatory role in the DDR and DNA repair, the quest turned rapidly into trying to identify specific TLK inhibitors to enhance the effectiveness of XRT or radiomimetic drugs. This led to the initial identification of certain phenothiazines (PTH) antipsychotics as surprisingly specific TLK inhibitors that in fact enhanced the killing of cancer cells, in vitro and xenografts, when combined to IR or doxorubicin [27]. A newer

PTH scaffold, called J54, that substantially lacked anti-dopaminergic undesirable effect, showed very promising results in the regression of androgen-sensitive prostate cancer cells largely by passing the DDR and thereby enforcing entry unto catastrophic mitotic progression, even resulting in substantial tumor regression in SCID xenografts [46]. Other studies of structure/function of TLKs have revealed additional potential inhibitors [47]. Considering the availability of such drugs, of even greater importance now, is the rebound effort to target the activity of TLK1 on RAD9 and RAD54, with the obvious suggestion that both translesion (TSL) repair, single-strand gaps, and HRR can be simultaneously targeted. Various chemotherapeutic agents, therefore, fall under the umbrella of TLK inhibitors for their therapeutic potentiation. These include for example: topoisomerase poisons, bleomycin, MMC, PARPis, and cisplatin, all of which ultimately lead to the formation of SSBs and DSBs. For some of these (e.g., doxorubicin and cisplatin) direct evidence of synthetic lethality in combination with TLK inhibitors has already been verified [27,48]. Inhibiting TLK1 prior to radiation in diseases like GBM where base-excision repair is activated [40] or cholangiocarcinoma or prostate cancer where intra-strand crosslink repair is elevated [49] increases their chemo-sensitization, which suggests that TLK1 forms nexus of a vast spectrum of DNA repair mechanisms (Figure 2). In addition to these obvious actionable targets via inhibitors of TLK1's role in DNA repair and checkpoint surveillance, more recent functions for TLK1 in disparate important oncogenic pathways, such as regulation of AKT (via AKTIP); the important pro-metastatic kinase MK5; and the ultimate effector of the Hippo pathway, YAP (via NEK1) are coming to light (rev. in [50]). In all the important roles of TLKs in various aspects of oncogenic development and as possible targets for various cancer-directed therapies is slowly but surely becoming 'untousled'.

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Conflicts of Interest: The authors declare that no conflicts exist.

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