Title: Understanding the mechanistic role of post translational modifications associated with TDP1 in response to DNA damage and anti-cancer agents

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One of the most common forms of DNA damage that arises in cells are single-strand breaks (SSBs), which can emerge from the abortive activity of DNA topoisomerase 1 (Top1), due to the covalent trapping of Top1 with the 3′-end of the DNA, leading to the generation of Top1-linked DNA covalent cleavage complexes (Top1cc). The key enzyme for the excision of Top1cc is Tyrosyl DNA Phosphodiesterase 1 (TDP1), which hydrolyzes the phosphodiester bond between the Top1-tyrosyl moiety and the DNA 3′-end. This dissertation discovers a novel phosphorylation of TDP1 at Ser 61 (S61) by the core mitotic regulatory kinase Cyclin dependent Kinase 1 (CDK1) through its direct binding with TDP1. CDK1-knockdown abrogated the endogenous pS61-TDP1 signal identified through TDP1-S61 specific phosphopeptide antibodies. TDP1-S61 phosphorylation favours TDP1 dissociation from the mitotic chromosomes. We show that replication stress induces pathological trapping of the phosphomutant S61A mutant-TDP1 on the mitotic chromosomes, particularly at the common fragile sites (CFSs), leading to severe mitotic defects. Mitotic DNA synthesis (MiDAS) is a break induced repair (BIR) mechanism that resolves late replication intermediates, thereby supporting cell proliferation under replication stress in mitosis. This unusual form of DNA synthesis uses Mus81-Eme1-SLX4, RAD52, and PolD3. This process can be detrimental if hyperactivated and can lead to chromosomal instabilities and segregation defects. We show unrestrained MiDAS in TDP1 deficient cells complemented with $TDP1^{S61A}$, which could be rescued by Mus81 knockdown. Tapping of $TDP1^{S61A}$ on mitotic chromosomes results in the formation of mitotic bridges, broken chromosomes, and micronuclei that are hallmarks of genomic instability. Our findings provide a novel insight into the cell cycle-dependent regulation of a primarily S-phase repair protein for safeguarding genomic instability. In the final chapter of this dissertation, we identified a novel Pyridine-Imidazo-Quinoline (PIQ) derivative that selectively poisons *Leishmania donaovani* DNA topoisomerase 1 (LdTop1) identified through screening of a Top1-targeted synthetic library. The novel PIQ derivative poisons LdTop1cc, leading to parasitic killing not only in the wild type *L. donovani* Ag83 strain but also the clinical isolates of antimony resistant *L. donovani* BHU575 strain, paving the way for the development of new anti-leishmanial agents to overcome drug resistance. Together, this dissertation provides a mechanistic insight into the generation and repair of tapped Top1cc across the phylogeny, from parasites to humans.

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