REVIEW

TDP1-dependent DNA single-strand break repair and neurodegeneration

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DNA single-strand breaks (SSBs) are the commonest DNA lesions that arise spontaneously in living cells. Cells employ efficient processes for the rapid repair of these breaks and defects in these processes appear to preferentially impact on the nervous system, causing human ataxia. Spinocerebellar ataxia with axonal neuropathy (SCAN1) is a human disease that is associated with a defect in repairing certain types of SSBs. Although it is a rare neurodegenerative disease, understanding the molecular basis of SCAN1 will lead to better understanding of the mechanisms that underpin not only neurodegeneration but also cancer.

DNA single-strand breaks and human ataxia

DNA is under continuous threat and thousands of DNA single-strand breaks (SSBs) arise in each cell per day (1). SSBs can arise either directly (e.g. from attack of deoxyribose by reactive oxygen species) or indirectly via enzymatic cleavage of the phosphodiester backbone during DNA base excision repair (2). Cells have employed different mechanisms to repair DNA SSBs, which are collectively termed DNA SSB repair (SSBR) (3,4). Defects in the repair of, or response to, DNA damage have been associated with many human disorders such as cancer, immunodeficiency and neurodegeneration. Recent data suggest that defects in the repair or response to DNA SSBs may have particular impact on the nervous system and are associated with human ataxia (5–7).

Ataxia is a neurological dysfunction of motor coordination that can affect gait, speech, gaze and balance (8). The first autosomal dominant gene for a hereditary ataxia, spinocerebellar ataxia (SCA1), was identified in 1993 (9). Autosomal dominant SCAs are caused mainly by a toxic gain-of-function mutations arising from an expansion of CAG-triplet repeats in the coding region of the disease gene, which results in the production of a mutant protein with an abnormal polyglutamine stretch (10). In contrast, autosomal recessive ataxias are often caused by loss-of-function mutations that result in perturbations in the normal control of, or response to, oxidative stress and/or DNA damage (8,11–14). Clinically, autosomal recessive SCAs are characterized by cerebellar ataxia and progressive degeneration of the cerebellum and spinocerebellar tract (11). Disorders in DNA repair typically cause additional symptoms such as mental retardation, photosensitivity, immunodeficiency and neoplasia (15,16). For example, two spinocerebellar ataxias with defects in response to DNA double-strand breaks (DSBs), ataxia telangiectasia (AT) and ataxia telangiectasia-like disorder (ATLD), also exhibit radiosensitivity, immunodeficiency and chromosomal instability (16–18).

Spinocerebellar ataxia with axonal neuropathy (SCAN1)

Recently Takashima et al. (7) identified an autosomal recessive ataxia, which they named spinocerebellar ataxia with axonal neuropathy (SCAN1). Interestingly, the SCAN1 phenotype lacks chromosomal instability and cancer predisposition, and symptoms appear to be restricted to the nervous system. SCAN1 is associated with a mutation in tyrosyl-DNA phosphodiesterase 1 (TDP1), a protein that is primarily involved in the repair of DNA strand breaks created by topoisomerase 1 (Top1). Top1 relaxes superhelical tension in DNA and during its normal catalytic cycle generates a reversible and transient intermediate known as the Top1 cleavage complex, in which Top1 is covalently attached via a tyrosyl residue (human Tyr723) to the 3'-terminus of a single-stranded nick (19). Following release of torsional stress, Top1 releases the nick and restores the integrity of the double helix (20). The rate of religation is normally much faster than the rate of cleavage and thus the steady-state concentration of these intermediates is very low (20,21). However, under certain circumstances, Top1 cleavage complexes may become irreversible, which is probably due to misalignment of the 5'-hydroxyl that is no longer able to act as a nucleophile in the religation reaction (20). Consequently, this irreversible ‘abortive’ Top1-associated DNA SSBs require a DNA repair process for its removal. For example, Top1 cleavage complexes can be converted into abortive Top1-associated DNA DSBs by collision with a replication fork (22–26). In addition, collision with the transcription machinery converts Top1 cleavage complexes into abortive Top1-associated DNA SSBs, and occasionally abortive Top1-associated DSBs if two Top1 cleavage complexes are closely spaced on opposite strands of DNA (27–29). Finally, some mutagenic DNA adducts (30–32) and endogenous DNA lesions such as abasic sites, nicks, gaps and DNA secondary structures can convert Top1 cleavage complexes into abortive Top1-associated DNA SSBs (Figure 1) (33–37).

Tyrosyl-DNA phosphodiesterase (TDP1)

In 1996, Nash and colleagues (38) employed a single-stranded DNA oligonucleotide linked to a tyrosine residue via a 3'-phosphoryl group, mimicking an abortive Top1-associated DNA strand break, to identify an activity in Saccharomyces cerevisiae extract that was able to remove Top1 from the 3'-terminus of DNA. They named the activity tyrosyl-DNA phosphodiesterase 1 (Tdp1), and gene encoding this activity was subsequently cloned (39). TDP1 is highly conserved in eukaryotes, with identifiable orthologs in a wide variety of animals, including humans (20,39–42). Sequence analysis of

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S. cerevisiae Tdp1 (39,43) and the crystal structure of human TDP1 (44,45) reveal it to be a member of the phospholipase D (PLD) superfamily. These enzymes contain two copies of a highly conserved sequence (HXK(X)4D(X)6GSXN), known as HKD motif (43) and catalyse phosphoryl transfer reactions. Despite lacking the aspartate conserved in other HKD motifs, the crystal structure of human TDP1 has shown that both histidine and lysine residues of the HKD motifs are clustered in the active centre of the enzyme (42,44,45).

Our initial understanding of the role for TDP1 in DNA repair came from studies conducted in budding yeast. Yeast Tdp1 provides one of several potentially redundant mechanisms for removing Top1 peptide from a DSB created by collision of Top1 cleavage complexes with DNA replication forks (46–48). Once Top1 peptide has been removed, the DSB is then channelled into the homologous recombination (HR) pathway for DSB repair (DSBR) (47,48). Tdp1 in yeast appears to operate in conjunction with HR to repair Top1-associated DSBs, because mutation of Tdp1 is epistatic to mutations in critical components of the HR pathway, such as Rad52 (47,48). As in yeast, it is possible that human TDP1 plays a role during DNA replication. The cytotoxic effect of the Top1-specific inhibitor camptothecin (CPT) is S-phase specific in most wild-type mammalian cell lines examined (49–52) and is ablated by co-incubation with aphidicolin, an inhibitor of DNA replication (23,25). Thus, ongoing replication forks appear to be required to convert Top1 cleavage complexes into cytotoxic lesions, in wild-type proliferating cells at least (53–60). It is therefore plausible that TDP1 inhibitors may enhance the anti-cancer activity of Top1 inhibitors and act as anti-proliferative agents (61). Consistent with a possible role for human TDP1 during replication, lymphoblastoid cell lines (LCLs) from affected SCAN1 individuals appear to show more late S-phase arrest than normal cells following CPT treatment (62). This could reflect a direct role for TDP1 during replication or an accumulation of un-repaired SSBs that are consequently converted into DSBs by collision with replication forks.

Nevertheless, a role for TDP1 during DNA replication is unlikely to account for the SCAN1 pathology, which appears to be restricted to post-mitotic neurons and lacks any obvious genetic instability or cancer. To address this question, we
recently examined the ability of SCAN1 LCLs to repair CPT-induced DNA strand breaks using the alkaline comet assay. SCAN1 cells accumulated more total DNA strand breaks than did normal cells during a 1 h incubation with CPT. These breaks failed to decline in SCAN1 cells during a subsequent incubation in CPT-free medium and approximately half were replication-independent and primarily comprised of DNA SSBs. The DNA breaks that accumulate in SCAN1 may arise from a stalled Top1 or could reflect a stalled TDP1 attempting to repair an abortive Top1 break. The latter possibility arose from the recent observation that TDP1 mutation in SCAN1 causes the accumulation of the TDP1–DNA covalent reaction intermediate (62). It is worth noting, however, that accumulation of CPT-induced DNA breaks is also observed in neural cells lacking TDP1 (El-Khamisy, S.F. and McKinnon, P.J. unpublished observations).

SCAN1 cells were also less able than normal cells to repair oxidative DNA SSBs induced by H$_2$O$_2$, though the defect was not as pronounced as for CPT (63). This could reflect a direct requirement for TDP1 in processing oxidative DNA breaks. It has been shown that TDP1 can remove glycolate from 3’-phosphoglycolate termini of DSBs (64) and a variety of other 3’-adducts from DNA (65). In addition, yeast expressing an active site mutant of Tdp1 (His182Ala) are hypersensitive to bleomycin, a radiomimetic agent which generates DNA breaks that are almost exclusively terminated with 3’-phosphoglycolate (66). Moreover, in vitro assays have recently shown that SCAN1 cell extracts lack any detectable processing of 3’-phosphoglycolate at single-stranded oligomers or at 3’-overhangs of DSBs (67). However, the use of a substrate with a single-stranded gap that harbours 3’-phosphoglycolate revealed APE1 as the major 3’-phosphoglycolate-processing activity in human cell extracts (68). This suggests that TDP1 may not be a major contributor in removing 3’-phosphoglycolate at sites of SSBs. An alternative explanation for the reduced repair of oxidative SSBs in SCAN1 cells would be an increased formation of Top1-associated SSBs following treatment with H$_2$O$_2$. Indeed, the presence of nicks (34), abasic sites (37), modified bases (69,70) and modified sugars (71) has been shown to stabilize and/or induce Top1 cleavage complexes. Consistent with this, Top1-deficient P388/CPT45 murine leukaemia cells are more resistant to H$_2$O$_2$ than Top1-proficient parental cells (72). In addition, over-expression of Top1 in yeast confers sensitivity to H$_2$O$_2$ and top1-mutant strains are more resistant to H$_2$O$_2$ than their isogenic wild-type strains (72). Finally, studies employing HeLa cells have confirmed that H$_2$O$_2$ does indeed induce Top1-DNA cross-links (72).

**TDP1 is a component of the SSBR machinery**

Yeast two-hybrid and co-immunoprecipitation experiments have provided evidence for a direct interaction between TDP1 and DNA ligase IIIα, a component of the DNA SSBR machinery (63). In agreement with this, it has been shown that TDP1 co-immunoprecipitates with XRCC1 in rodent cell extracts (73). The interaction between TDP1 and DNA ligase IIIα is mediated by the N-terminal domain of TDP1, a region that is poorly conserved or absent from Tdp1 of lower eukaryotes (43,63). Budding yeast lack orthologs of various components of SSBR machinery found in mammals, including XRCC1 and DNA ligase IIIα. Therefore it seems likely that the N-terminal domain of human TDP1 may have evolved to couple this enzyme to the SSBR machinery, through an interaction with DNA ligase IIIα. Interestingly, it has been shown that the global SSBR process that operates throughout interphase requires the BRCTII domain of XRCC1, a domain that mediates the interaction with DNA ligase IIIα (74,75). The observation that TDP1 interacts with DNA ligase IIIα and that TDP1 mutation is manifested in non-proliferating cells is thus consistent with a particular importance of the SSBR process in post-mitotic tissue.

The current models of SSBR suggest sequential recruitment of the enzymes such that DNA repair-intermediates are passed from one enzyme to the other in a molecular relay (76,77). However, at least some of the XRCC1 appear to be present in pre-formed multi-protein complexes (78–81). TDP1 appears to be constitutively associated with SSBR machinery, though it is unclear whether these interactions are occurring in response to ‘endogenous’ levels of DNA damage or are truly constitutive.

**Why does mutation of TDP1 cause neurodegeneration?**

The brain encounters high levels of oxidative stress as it consumes ~20% of the inhaled oxygen and possesses low levels of antioxidant enzymes (82). The formation of high levels of oxidative lesions may directly require TDP1-SSBR for their removal and may also trap more Top1, which in turn increases the demand for TDP1-SSBR. In addition, there is a high level of transcriptional demand in post-mitotic neurons. For example, cortical neurons incorporate ~5.5-fold more $^3$H-uridine than astrocytes (83) and possess twice the number of transcription initiation sites as compared to glial cells (84–86). It is thus possible that the high transcriptional activity in neurons may increase the frequency of collision between RNA polymerases and un-repaired SSBs, which can consequently block elongating RNA polymerases and compromise cellular function. Consistent with this, SCAN1 cells were unable to recover transcription following CPT treatment (63). The absence of any obvious genetic instability or cancer in SCAN1 may reflect the ability of homologous recombination (HR) repair to compensate for defective TDP1-SSBR by removing un-repaired SSBs during DNA replication. Consistent with this, HR is selectively elevated in SCAN1 cells as indicated by an increased frequency of ‘spontaneous’ and ‘CPT-induced’ sister chromatid exchanges (63). Thus, it seems possible that HR can fulfill a back-up role in the absence of TDP1-dependent SSBR, allowing proliferating SCAN1 cells to tolerate un-repaired SSBs, at physiological levels of DNA damage at least (Figure 2). If this is true, then why are other predominantly non-cycling tissues not affected in SCAN1? The high levels of oxidative stress and transcriptional demand in post-mitotic neurons, coupled to the limited regenerative capacity of the nervous system, may render this tissue particularly susceptible to defects in TDP1-SSBR and the possible cell loss. This is in contrast to cell loss from tissues with greater regenerative capacity that may be better tolerated. For example, myocytes and adipocytes are also terminally differentiated, but can be replaced by precursor cells (87–89).

**Conclusion and future directions**

Whereas a great insight into the molecular role of TDP1 has come from studies with yeast, SCAN1 cells and recombinant TDP1, there is still much that is unresolved. To date, most studies have utilized transformed cells, but a demonstrated role for TDP1 in primary neuronal cells is currently lacking. Accordingly, we are only beginning to understand the possible
impact of un-repaired SSBs on post-mitotic neuronal cells and many issues remain. For example, why might TDP1 mutation selectively affect certain neuronal tissue such as the cerebellum? Why is the developing brain resistant to TDP1 mutation and the disease has late onset? Does mutation in other components of SSBR machinery such as DNA ligase IIIa and XRCC1 also impact on the nervous system? The development of animal models and conditional knockout alleles will be an important tool to address these questions and to enhance our understanding of the relationship between SSBR and neurodegeneration.

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