




Transcription-dependent DNA double-strand breaks and human disease

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ABSTRACT

Accumulation of DNA damage in resting cells is an emerging cause of human disease. We identified a mechanism of DNA double-strand break (DSB) formation in non-replicating cells, which strictly depends on transcription. These transcriptional DSBs arise from the twinned processing of R-loops and topoisomerase I and may underlie neurological disorders and cancers.

ARTICLE HISTORY

Received 29 October 2019
Revised 8 November 2019
Accepted 8 November 2019

KEYWORDS

DNA double-strand break;
DNA repair; R-loop; RNA/
DNA hybrid; Senataxin;
TDP1; XPF;
Neurodegenerative disease;
Cancer; Topoisomerase I;
Transcription

DNA double-strand breaks (DSBs) are infrequent but among the most harmful genomic lesions. Their defective repair can induce genomic rearrangements, mutations or cell death, and they have been implicated in the pathogenesis of several human diseases, including neurodegenerative syndromes and cancers. In dividing cells, DSBs occur primarily during DNA replication. Hence, their mechanisms of formation have been well documented, such as in the events of replication of a damaged DNA template and during conflicts between replication and transcription machineries. However, in human, most cells are non-replicating, and increasing evidence indicates that DNA damage and genomic instability in such cells can cause disease. Accumulation of DNA breaks in post-mitotic neurons is the underlying cause of multiple neurodegenerative syndromes.¹ Many solid tumors are also characterized by a subpopulation of quiescent cells, which represents a fuel for cancer diversity and evolution.² Currently, very little is known about how DSBs are generated in non-replicating cells, even though such knowledge is likely to provide information on the aetiology of several human diseases.

Our recent work has advanced this field by identifying a mechanism for DSB formation in non-replicating cells occurring under both physiological and pathological conditions.³ DSBs arise from two nearby single-strand breaks (SSBs) on the opposing DNA strands, both produced during transcription. One SSB results from the repair of a transcription-blocking topoisomerase I cleavage complex (TOP1cc), and the other from the cleavage of an R-loop structure (Figure 1). TOP1 removes DNA superhelical tensions generated during transcription by producing transient TOP1ccs.⁴ We found that stabilization (trapping) of TOP1ccs on chromatin primes the formation of DSBs by blocking transcription to promote R-loop formation. Notably, TOP1ccs are often trapped by DNA modifications, such as oxidative base damage,⁴ and they are associated with human disease (Figure 1). The repair of TOP1ccs generates SSB intermediates, and we found that DSBs

are produced by concurrent SSBs on the opposing DNA strand of the R-loops. R-loops are RNA/DNA hybrids with displaced single-stranded DNA. They are widespread structures that can play physiological roles, but their unscheduled formation is a source of DNA breaks.⁵ Recent work reported the involvement of xeroderma pigmentosum complementation group F (XPF, also known as ERCC4) and group G (XPG, also known as ERCC5) flap nucleases in the production of R-loop-dependent DSBs in replicating cells.⁶ Our study in non-replicating cells sheds new light on R-loop processing by nucleases.³ It identified that flap structure-specific endonuclease 1 (FEN1), XPF and XPG induce SSBs in the R-loops by the cleavage of one strand and the DSB is created when another SSB is present on the opposing DNA strand. Our study further demonstrated that the single-stranded DNA in the R-loop must be cleaved at both 3'- and 5'-extremities to induce DSBs and that this dual incision is mediated by XPF/XPG or XPF/FEN1. However, it is still unclear what defines the choice between these specific combinations of nucleases.

These transcriptional DSBs have been detected in post-mitotic neurons⁷ and quiescent cells,³ and may underlie human disease. Indeed, we found that genetic defects in TOP1cc removal pathway [tyrosyl-DNA phosphodiesterase 1 (*TDP1*), polynucleotide kinase 3'-phosphatase (*PNKP*), or X-ray repair cross-complementing 1 (*XRCC1*)] or in R-loop resolution [senataxin (*SETX*)] enhance transcriptional DSBs in non-replicating cells.³ Notably, all these genetic defects cause neurological disorders, primarily cerebellar ataxia and amyotrophic lateral sclerosis (Figure 1), suggesting that these transcriptional DSBs may be the underlying cause of several neurodegenerative syndromes. This is further supported by the observation that numerous other genetic (or protein) defects that act to increase TOP1ccs [ataxia telangiectasia mutated (*ATM*), DNA-dependent protein kinase (DNA-PK), or chromosome 9 open reading frame 72 (*C9orf72*) expansion

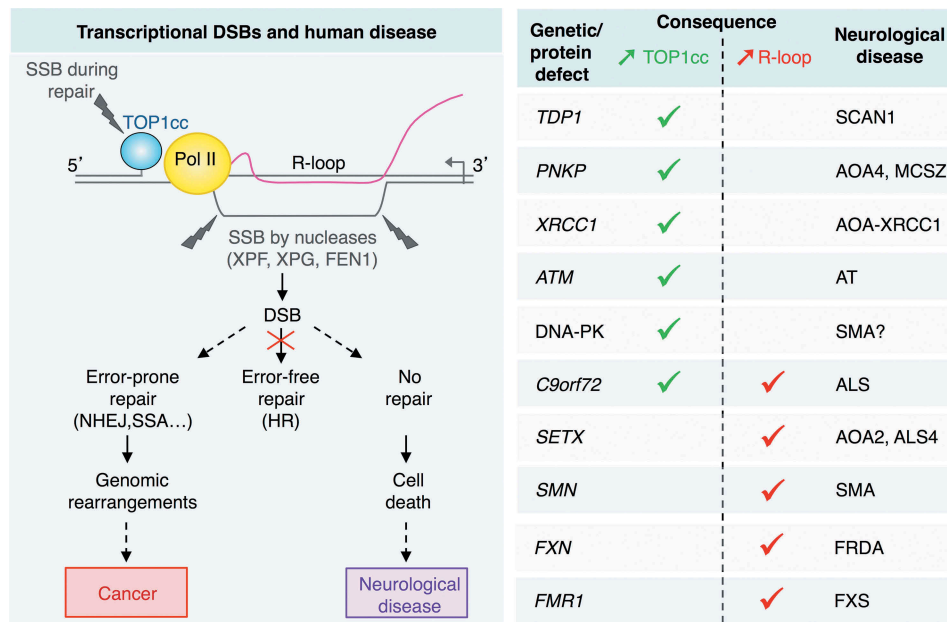


Figure 1. Outcomes of transcription-dependent DNA double-strand breaks (DSBs) in non-replicating cells. DSBs arise from two single-strand breaks (SSBs) on the opposing DNA strands: one SSB results from repair of a transcription-blocking topoisomerase I cleavage complex (TOP1cc), and the other from the cleavage of an R-loop by nucleases. In non-replicating cells, error-free homologous recombination (HR) is unavailable. Hence, DSBs could be repaired by error-prone pathways, such as non-homologous end joining (NHEJ) and single-strand annealing (SSA), which may promote genomic rearrangements and mutations. If left unrepaired, transcriptional DSBs may accumulate and induce cell death. The right-hand side table shows human neurological disorders, the genes (or protein expression) altered in these diseases and their association with increased TOP1ccs (green) or R-loops (red). AOA: ataxia with oculomotor apraxia; ALS: amyotrophic lateral sclerosis; AT: ataxia telangiectasia; *ATM*: ataxia telangiectasia mutated; *C9orf72*: chromosome 9 open reading frame 72; DNA-PK: DNA-dependent protein kinase; FEN1: flap structure-specific endonuclease 1; FRDA: Friedreich's ataxia; *FMR1*: fragile X mental retardation 1; *FXN*: frataxin; FXS: fragile X syndrome; MCSZ: microcephaly, seizures, and developmental delay; *PNKP*: polynucleotide kinase 3'-phosphatase; Pol II: RNA polymerase II; SCAN1: spinocerebellar ataxia with axonal neuropathy-1; *SETX*: senataxin; SMA: spinal muscular atrophy; *SMN*: survival motor neuron 1; *TDP1*: tyrosyl-DNA phosphodiesterase 1; XPF: xeroderma pigmentosum complementation group F; XPG: xeroderma pigmentosum complementation group G; *XRCC1*: X-ray repair cross-complementing 1.

repeat], and/or R-loops [*C9orf72*, frataxin (*FXN*) or fragile X mental retardation 1 (*FMR1*) expansion repeats, or survival motor neuron 1 (*SMN*)], are also associated with neurological disorders (Figure 1). A key question is why do these transcriptional DSBs primarily affect neuronal cells? First, neurons may be particularly prone to produce these breaks considering their high metabolic and transcriptional activity, which may promote reactive oxygen species (ROS)-dependent TOP1cc trapping⁴ and R-loop accumulation.³ Second, post-mitotic neurons are non-dividing cells, and as such have reduced repair capability compared to dividing cells. Homologous recombination (HR) and non-homologous end joining (NHEJ) are the main pathways for the repair of DSBs. HR is the preferred pathway to repair DSBs occurring in transcribed regions,⁸ however it is unavailable in non-dividing cells due to absence of sister chromatids for recombination (Figure 1). Our work further highlights that genetic defects in TOP1cc removal (*TDP1*) or R-loop resolution (*SETX*) prevent the repair of transcriptional DSBs in non-replicating cells.³ This raises the possibility that in neurons, transcriptional DSBs accumulate over time due to enhanced production and defective repair. Both will contribute to the neurodegenerative phenotype. Consistent with this, we found that such DSBs can kill non-replicating cells.³

It is conceivable that the transcriptional DSBs that depend on R-loops and TOP1ccs may contribute to other pathologies, including cancers. Indeed, many tumors contain a subpopulation of quiescent cells,² and increasing evidence indicates that genetic

alterations in cancer cells are linked to R-loop formation⁹ and TOP1 activity.⁴ Notably, many cancers have an altered cell metabolism resulting in increased ROS levels,¹⁰ which may further trap TOP1ccs,⁴ and an altered expression/mutation of many R-loop resolving factors [e.g. *SETX*, DExH-box helicase 9 (*DHX9*), breast cancer 1 and 2 (*BRCA1* and *BRCA2*), fanconi anemia (*FA*) proteins],⁹ which may further stabilize R-loops. In quiescent cancer cells, transcriptional DSBs may promote tumor heterogeneity and progression because they occur in transcribed regions and they can't use error-free HR repair. Their repair may rely on error-prone pathways, which may lead to mutations and genomic rearrangements (Figure 1).

Altogether, our work provides a new paradigm for the occurrence of DNA damage and genomic instability in resting cells in the context of human disease.

Acknowledgments

OS lab is supported by the Fondation pour la Recherche Médicale (FRM) [Equipe labellisée FRM (DEQ20170839117)] and the Ligue Nationale Contre le Cancer (LNCC) Comité Départemental 31. NG lab is supported by the Royal Society University Research fellowship. AC is supported by the EPA Research Fund (Sir William Dunn School of Pathology, University of Oxford) and Ataxia UK grant to NG. We thank Nick Proudfoot for critically reading the manuscript.

Disclosure of Potential Conflicts of Interest

The authors declare no conflict of interest

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