



DNA Double Strand Breaks and Chromosomal Translocations Induced by DNA Topoisomerase II

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DNA double strand breaks (DSBs) are the most cytotoxic lesions of those occurring in the DNA and can lead to cell death or result in genome mutagenesis and chromosomal translocations. Although most of these rearrangements have detrimental effects for cellular survival, single events can provide clonal advantage and result in abnormal cellular proliferation and cancer. The origin and the environment of the DNA break or the repair pathway are key factors that influence the frequency at which these events appear. However, the molecular mechanisms that underlie the formation of chromosomal translocations remain unclear. DNA topoisomerases are essential enzymes present in all cellular organisms with critical roles in DNA metabolism and that have been linked to the formation of deleterious DSBs for a long time. DSBs induced by the abortive activity of DNA topoisomerase II (TOP2) are “trending topic” because of their possible role in genome instability and oncogenesis. Furthermore, transcription associated TOP2 activity appears to be one of the most determining causes behind the formation of chromosomal translocations. In this review, the origin of recombinogenic TOP2 breaks and the determinants behind their tendency to translocate will be summarized.

Keywords: DSB repair, DNA topoisomerase II, chromosomal translocations, genome instability, transcription

INTRODUCTION

Chromosomal translocations are rearrangements of large fragments of DNA. When transcribed regions are affected, genome translocations usually result in the inactivation of one or a group of genes with the consequent deleterious effects for cellular survival. However, on occasion, translocations can generate chimeric proteins or deregulate transcription programmes creating abnormal growth capacities and contributing to malignancy and tumor development. Over 10,000 gene fusions have been found in cancer, most of which are considered passenger mutations, consequence of the intrinsic instability of tumor progression. Among them, more than 300 are recurrent and contribute to initial stages of cellular disarray (Mitelman et al., 2007; Mertens et al., 2015).

Recurrence of chromosomal translocations is determined by a large number of factors, starting from the nature of the DNA break and including the pathway involved in its repair, the cell cycle stage, the chromatin status of the locus, and the genomic location of the lesion. Since most of these factors are dynamic and interconnected, their relative relevance is difficult to establish, and many aspects of the origin of genomic translocations remain unclear. Recent studies have unveiled that transcription and 3D organization of the genome are two major determinants in the appearance

of DNA double-stranded breaks (DSBs) and they promote chromosomal translocations. In this brief review how DNA topoisomerase II (TOP2) appears at the crossroad of these factors will be discussed.

DNA TRANSACTIONS AND TOP2 ACTIVITY

DNA topoisomerases are essential enzymes present in all cellular organisms with critical roles in DNA metabolism. DNA topoisomerases release the torsional stress generated in the DNA by a wide variety of processes including replication, transcription, 3D genome organization, and chromosome segregation (Pommier et al., 2016). According to their mechanism of action, DNA topoisomerases are classified in two types depending on whether they cut one (type I) or two strands (type II) of the DNA double helix. TOP2 is a type II enzyme that can pass an intact DNA duplex through a broken one while covalently bound to the DNA. Once strand passage is completed, the enzyme reseals the break (Nitiss, 2009a). Vertebrates express two TOP2 isoforms, TOP2 α and TOP2 β . While TOP2 β is expressed throughout the cell cycle, TOP2 α levels correlate with cellular proliferation and peak at S and G2/M phases. TOP2 α has a major role in replication and chromosome segregation. TOP2 β activity has been mainly associated to transcription. It participates in: transcription elongation, conserving the structure of either active or inactive promoters, promoting the activation of hormone-driven, and early response genes and in the release of paused RNA polymerases (Ju, 2006; Haffner et al., 2010; Madabhushi et al., 2015; Dellino et al., 2019).

A key intermediate of topoisomerase activity is the cleavage complex (TOP2cc), formed when the topoisomerase cleaves the DNA and each subunit of the TOP2 dimer becomes covalently linked to the 5'-terminus of the break via a phosphotyrosyl bond (Vos et al., 2011) (Figure 1). Although the cleavage complex is normally transient, naturally due to unclear circumstances or induced by the presence of anti-tumor agents that act as topoisomerase "poisons" the cleaved intermediate can result in the formation of abortive (irreversible) TOP2cc, a singular DSB (Deweese and Osheroff, 2009; Nitiss, 2009b).

TOP2 AND ONCOGENIC TRANSLOCATIONS

TOP2-associated translocations are main drivers of some common hematological and solid tumors (Felix et al., 2006; Haffner et al., 2010). Oncogenic translocations related to TOP2 have been mainly associated to TOP2 β activity (Nitiss, 2009a; Pommier et al., 2016; Madabhushi, 2018). However, after many years of study, we only start to understand the molecular mechanisms that direct TOP2-induced rearrangements.

In prostate cancer, androgen-regulated genes are frequently fused to transcription factors of the ETS family. For instance, the fusion of *TMPRSS2* and *ERG* occurs in more than 50% of prostate malignancies resulting in a hormone-dependent expression of *ERG* in prostate tissue (Kumar-Sinha et al., 2008). *TMPRSS2* and *ERG* expression has been linked to TOP2 activity since TOP2 β

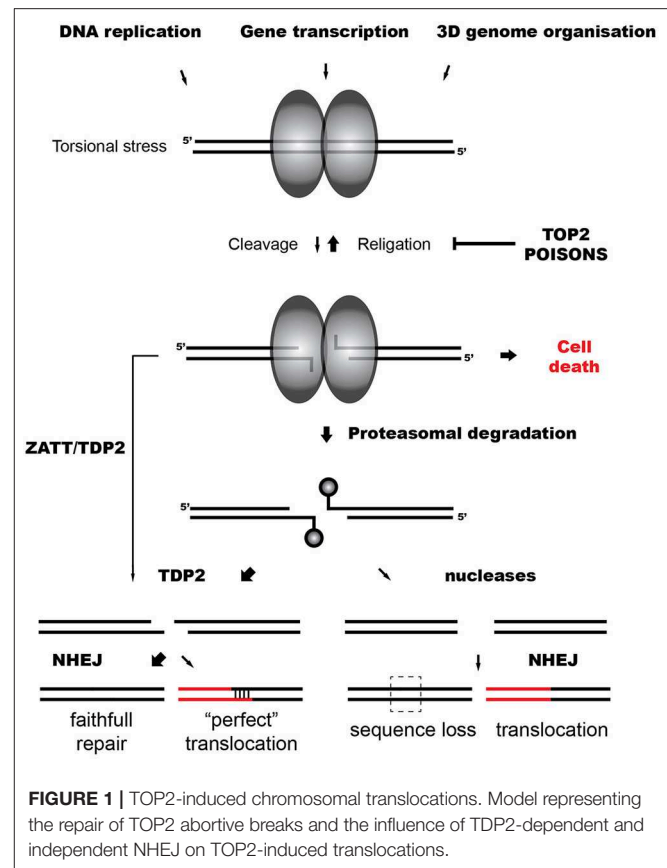


FIGURE 1 | TOP2-induced chromosomal translocations. Model representing the repair of TOP2 abortive breaks and the influence of TDP2-dependent and independent NHEJ on TOP2-induced translocations.

participates in the androgen-dependent activation of these genes. Androgen signaling promotes co-recruitment of androgen receptor and TOP2 β to *TMPRSS2* and *ERG* breakpoints, which can trigger recombinogenic DSBs (Haffner et al., 2010).

Recurrent fusions involving *MLL* and members of the super-elongation complex, such as *AF4* and *AF9*, account for 10–30% of secondary and infant acute myeloid leukemia (AML) (Mitelman et al., 2007; Mertens et al., 2015). Numerous potential mechanisms for *MLL* breakage have been proposed, from Alu-mediated recombination to TOP2 β -induced breaks (Cowell and Austin, 2012; Wright and Vaughan, 2014). Notably, secondary leukemias are those resulting from the use of genotoxic chemotherapeutic drugs, mainly alkylating agents or TOP2 inhibitors, uplifting the direct connection between TOP2 and translocations in *MLL* (Wright and Vaughan, 2014). The link of infant leukemia with TOP2 abortive activity is less clear but a correlation with dietary flavonoids, natural TOP2 poisons, has been proposed (Ross, 2000).

THE CONTRIBUTION OF TOP2 IN THE CELLULAR POOL OF DSBs

The first factor influencing the propensity of a region to translocate is the frequency of DNA breakage. DSBs can arise directly from exogenous threats (clastogens), such as radiation and chemotherapeutic or industrial chemicals. Endogenous

threats are stochastic activity of apoptotic caspases, nucleases such as RAG1 and RAG2, and TOP2 (Ashour et al., 2015; Lieber, 2016). DSBs can also form indirectly from coincident single strand breaks (SSBs), induced exogenously by alkylating chemotherapeutic agents, or naturally by type I DNA topoisomerases, reactive oxygen species (ROS), or activation-induced cytidine deaminase (AID) (Xu et al., 2012; Rulten and Caldecott, 2013). DNA replication across SSBs also generates single ended DSBs (Kuzminov, 2001). This is a prominent source of DSBs, since SSBs are known to be as frequent as 50,000 per day per cell (Lindahl, 1993). Finally, replication stress, due to replication fork encountering with inter-strand crosslinks or non-B forms of DNA such as RNA-DNA hybrids (R-loops), is also known to promote DSB formation (Gómez-González and Aguilera, 2019).

The most precise information about endogenous DSBs comes from non-biased DSB mapping methods, developed to evaluate illegitimate cleavage by RAG nucleases, and AID in B cells or by CRISPR-Cas (Chiarle et al., 2011; Crosetto et al., 2013; Tsai et al., 2014; Frock et al., 2015; Canela et al., 2016; Lensing et al., 2016; Yan et al., 2017). These studies draw two major conclusions. The first one is that recurrent translocations (typically those that drive specific cancers) are mostly tissue-specific and triggered by recurrent DSBs. For instance, RAG off-target sites have been efficiently detected in activated mouse B-cells in which Rag1 and Rag2 are induced, supporting the role of stochastic activity of these nucleases in the formation of B-cell specific DSBs (Kuo and Schlissel, 2009; Chiarle et al., 2011; Canela et al., 2016).

The second conclusion of these studies, and probably the most ground-breaking, is that there are more stochastic sources of DSBs that are not cell-cycle nor tissue-specific but can be consistently detected in mice and human cells. Some of these are related to replication stress and frequently appear in long gene bodies, which are prone to undergo late replication and are predisposed to replication-transcription conflicts (Canela et al., 2016; Wei et al., 2016). Notably, others, a bulky group of them (over 60%), increase in frequency in the presence of the TOP2 poison etoposide (Canela et al., 2017). These breakpoints, concentrate in chromatin loop boundaries, gene bodies and promoter-proximal locations, frequently transcription start sites (TSS) (Chiarle et al., 2011; Schwer et al., 2016; Yan et al., 2017).

But, what is the origin of these DSBs? TOP2 β is positioned at loop anchors, this is, CTCF/cohesin (RAD21) binding sites that flank topologically associating domains, suggesting that it might be required to solve topological problems during loop extrusion dynamics (Uusküla-Reimand et al., 2016). Etoposide-induced TOP2cc can be detected in these loci, independently of transcription and replication activities (Canela et al., 2017). But these are reversible TOP2cc. Contrary, detection of abortive TOP2cc (irreversible) by DSB or protein-linked mapping has demonstrated that the induction of DSBs at loop anchors by TOP2 activity is largely depend on active transcription (Canela et al., 2019; Gittens et al., 2019; Gothe et al., 2019). In fact, a large number of TOP2 β -associated breaks also concentrate in gene bodies and around TSS, independently of RAD21 (Chiarle et al., 2011; Schwer et al., 2016; Canela et al., 2017; Yan et al., 2017; Gittens et al., 2019; Gothe et al., 2019). Importantly,

distribution of TOP2 breaks around TSS positively correlates with transcription levels at these loci (Gittens et al., 2019). Indeed, the inhibition of transcription elongation prevents TOP2 breakage at these loci suggesting that transcription is a major driving force in TOP2 abortive cycles (Gómez-Herreros et al., 2017; Gothe et al., 2019). For instance, breaks at TSS associate with promoter fragility suggesting that events such as RNA polymerase II pause release requires TOP2 activity and is a source of DSBs (Dellino et al., 2019).

Regarding TOP2 isoforms, both TOP2 α and TOP2 β influence DNA breakage at these hotspots (Yu et al., 2017; Gothe et al., 2019). Intriguingly, and despite a similar localization of both isoforms, TOP2 β -lacking cells reduce breakage at these loci, suggesting a dominant role of TOP2 β over TOP2 α (Cowell et al., 2012; Canela et al., 2017).

In theory, any DSB can be a potential origin of a rearrangement. Interestingly, oncogenic breakpoints such as those found in *TMPRSS2*, *ERG*, *MLL*, *AF4*, and *AF9*, among many others, are localized to TOP2 β /CTCF/RAD21 breakpoints (Canela et al., 2017, 2019; Gothe et al., 2019). Moreover, TOP2-induced breaks have been detected by high-throughput, genome-wide translocation sequencing (HTGTS). HTGTS “fish” breaks genome wide using a bait DSB in a controlled locus (Chiarle et al., 2011; Frock et al., 2015). HTGTS has revealed the tendency of TOP2-induced breaks to translocate, with highly transcribed genes translocating more than with non-transcribed ones (Chiarle et al., 2011; Wei et al., 2016; Canela et al., 2019). Some of these hotspots are localized in TSS (Schwer et al., 2016).

ILLEGITIMATE TOP2 DSB REPAIR

The illegitimate repair of DNA ends *in trans* is mediated by DNA repair pathways, but how often and why are breaks incorrectly joined is not clear. The two major pathways involved in the repair of DSBs in eukaryotic cells are non-homologous end joining (NHEJ) and homologous recombination (HR). HR occurs specifically in late S and G2 phases of the cell cycle, as it requires the presence of a sister chromatid for the repair process (Mehta and Haber, 2014; Wright et al., 2018). HR is considered an error-free pathway due to the fact that a very large homology, up to megabases, is used, ensuring the accuracy of the repair (Symington, 2016). The occurrence of recombination between homologous chromosomes or tandem repeats has been shown to be substantially low and HR-deficient cells exhibit higher rates of genome rearrangements, historically absolving HR for almost any responsibility in chromosomal translocations (Moynahan and Jasin, 1997, 2010; Lambert et al., 1999; Stark and Jasin, 2003). Exceptions to the HR paradigm are Rad51-independent but homology-directed pathways, Break-Induced Replication (BIR) and single strand annealing (SSA), which can promote exchanges *in trans* (Elliott et al., 2005; Malkova and Ira, 2013; Bhargava et al., 2016; Sakofsky and Malkova, 2017; Kramara et al., 2018).

In contrast to HR, NHEJ is active throughout the cell cycle and involves the efficient ligation of DNA ends with minimal processing at the site of joining. NHEJ is considered

an error-prone pathway since cellular nucleases trim DNA ends to make them compatible before ligation (Lieber, 2010). The homology required in this route is reduced to 1–2 nucleotides, in case there is any, making NHEJ the ideal scapegoat to provoke illegitimate joinings (Chang et al., 2017). However, in the absence of KU70/80 or XRCC4-LIG4, core factors of canonical NHEJ (cNHEJ), a genetically-different, alternative NHEJ (altNHEJ) pathway takes over (Yan et al., 2007). altNHEJ is characterized by a longer homology requirement at the site of break that goes up to 10–20 bp (McVey and Lee, 2008). Resection is mediated by CtIP and the MRN complex, similarly to initial stages of HR (Zhang and Jasin, 2010; Ghezraoui et al., 2014). Base pairing *in trans* of these ends results in translocations characterized by short microhomologies (Guirouilh-Barbat et al., 2004; Kent et al., 2015; Mateos-Gomez et al., 2015; Sfeir and Symington, 2015; Zahn et al., 2015).

In the case of TOP2-induced DSBs, trapped TOP2 represents a particular barrier for ligation, and DNA ends need to be processed. Abortive TOP2cc are denatured and degraded by the proteasome, leaving a TOP2-derived peptide of unknown length covalently bound to the 5' phosphate of the DNA through a tyrosine residue (Zhang et al., 2006; Lin et al., 2008) (**Figure 1**). This protein adduct is a hallmark of TOP2 breaks and, like other blocking lesions, can condition DSB repair (Álvarez-Quilón et al., 2014). Resection can generate proficient substrates for HR independently of the nature of the DNA end and potentially remove these adducts. In accordance, HR-deficient cells are hypersensitive to TOP2 poisons, suggesting that TOP2 breaks can be repaired by HR when available (Gómez-Herreros et al., 2013). However, remaining topoisomerase can be precisely removed by Tyrosyl DNA phosphodiesterase 2 (TDP2), which cleaves the phosphotyrosyl bond between the tyrosine and the 5' phosphate of the DNA (Cortés-Ledesma et al., 2009; Zeng et al., 2010). TDP2 can also remove non-degraded TOP2 in a proteasome-parallel route stimulated by the SUMO-ligase ZATT (Schellenberg et al., 2017). Once TOP2 is removed by TDP2, remaining four base-pair cohesive overhang is ready to be ligated by cNHEJ (Gómez-Herreros et al., 2013) (**Figure 1**).

TDP2-mediated pathways protect cells from TOP2 abortive activity, accelerating TOP2 DSB repair and preventing cell death and genome instability induced by TOP2 poisons (Gómez-Herreros et al., 2014, 2017; Zagnoli-Vieira et al., 2018). In accordance, breaks in *MLL* induced by the abortive activity of TOP2 during transcription accumulate in cells lacking TDP2 (Gómez-Herreros et al., 2017). Notably, TDP2 facilitates a faithful repair of TOP2 breaks suppressing chromosomal translocations generated by TOP2 during transcription (Gómez-Herreros et al., 2017) (**Figure 1**). Intriguingly, TDP2-processed ends can also originate chromosomal translocations. A four base pair homology at break joining can be generated in cells treated with etoposide and is dependent on TDP2 (Gómez-Herreros et al., 2017) (**Figure 1**). About 20% of secondary AML is characterized by this type of junction that is referred to as “perfect” (Whitmarsh et al., 2003; Meyer et al., 2005, 2017). A very high number of DSBs might challenge physiological repair capacities and promote this illegitimate pairing.

Since TDP2 prevents genome instability and chromosomal translocations, it has been hypothesized that marginal routes would promote them (Caldecott, 2012; Gómez-Herreros et al., 2013, 2017). If HR is not available, in contrast to the “clean” end processing mediated by TDP2, endonucleases would potentially generate the loss of information at DNA ends (**Figure 1**). However, it has been shown that MRE11, the nuclease activity of the MRN complex, can process abortive TOP2 DSBs regulated by a HR-independent role of BRCA1 (Hoa et al., 2016; Sasanuma et al., 2018). Notably, MRE11 H129N (nuclease deficient) mutants exhibit increased instability and translocations when treated with TOP2 poisons (Sasanuma et al., 2018; Gothe et al., 2019). The contribution of MRE11 and other nucleases such as ARTEMIS in the repair of physiological levels of TOP2 breaks, their relevance in TOP2 poison-based chemotherapy and their implication in TOP2-induced genome instability is under discussion.

Contrary to mouse cells, in which translocations depend mostly on altNHEJ, cNHEJ mediates translocations induced by nucleases and ionizing irradiation in humans (Ghezraoui et al., 2014; Biehs et al., 2017; So and Martin, 2019). However, it has also been shown that the DSB structure can predispose repair toward cNHEJ and altNHEJ suggesting that the nature of the DNA end can condition its repair (So and Martin, 2019). The role of NHEJ in the formation of TOP2-induced translocations is controversial. An epistatic effect of Ku70 over TDP2 in etoposide sensitivity in avian cells suggests that cNHEJ mediates TOP2-induced DSB repair (Gómez-Herreros et al., 2013). However LIG4 deficiency increases *MLL* translocations suggesting that different pathways such as altNHEJ might mediate TOP2 induced rearrangements in the absence of cNHEJ (Gothé et al., 2019). Further research is required to clarify this point.

Noteworthy, despite in the presence of a sister chromatid NHEJ still has a dominant role (Beucher et al., 2009; Karanam et al., 2012), it has been shown that transcriptionally-active regions are preferentially repaired by HR, promoted by open chromatin marks (Aymard et al., 2014; Pfister et al., 2014). This mechanism may moderate mutagenic pathways during the repair of critical sequences. Why TOP2-induced DSBs during transcription are so dependent on TDP2 and NHEJ remains unknown.

THE SYNOPSIS OF TOP2 BREAKS

A major determinant for translocation propensity is the proximity between donor and acceptor DSBs (Roukos et al., 2013; Hu et al., 2016). Translocations occur preferentially *in cis* and are enhanced within the same topological domain due to pre-existing spatial proximity (Zhang et al., 2012). Notably, transcription may not only mediate TOP2 breakage but break proximity as well. Oncogenic translocation partners are known to share transcription factories (discrete concentrations of actively transcribed genes) (Ghamari et al., 2013). That is the case for *IgH* and *MYC* in Burkitt's lymphoma but also for TOP2 hotspots such as *TMPRSS2* and *ERG* in prostate and *MLL*, *AF4*, and *AF9* in bone marrow (Osborne et al., 2007; Lin et al., 2009; Cowell et al., 2012). Nevertheless, for other pairs such as *MLL* and *ENL*,

an inherent proximity exists, favoring synapsis independently of transcription (Gothe et al., 2019). The association of TOP2 breaks to loop anchors might also contribute to spatial proximity. However, a rational comparison of the 3D architecture of the genome with the genome-wide data of TOP2 abortive breaks is still missing.

Additionally, in G1, repair in highly transcribed loci is delayed and breaks dynamically cluster (Aten et al., 2004). The functional reason for this grouping remains unclear but the absence of a sister chromatid and the preferential use of HR over NHEJ would be an explanation (Aymard et al., 2017). However, grouping unrepaired, and may be partially resected, DSBs seems counter-productive for genome stability. Favoring TDP2-mediated repair in TOP2 breaks associated to transcription may be a mechanism to prevent this situation.

CONCLUSION

High transcription and recurrent DSBs are hallmarks of oncogenic hotspots. These two factors get together with spatial

proximity and NHEJ-mediated repair in transcription-associated TOP2 breaks generating the perfect breeding ground for chromosomal translocations.

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FG-H conceived this review and wrote the manuscript.

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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