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Topoisomerase II and leukemia

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Abstract

Type II topoisomerases are essential enzymes that modulate DNA under- and overwinding, knotting, and tangling. Beyond their critical physiological functions, these enzymes are the targets for some of the most widely prescribed anticancer drugs (topoisomerase II poisons) in clinical use. Topoisomerase II poisons kill cells by increasing levels of covalent enzyme-cleaved DNA complexes that are normal reaction intermediates. Drugs such as etoposide, doxorubicin, and mitoxantrone are frontline therapies for a variety of solid tumors and hematological malignancies. Unfortunately, their use is also associated with the development of specific leukemias. Regimens that include etoposide or doxorubicin are linked to the occurrence of acute myeloid leukemias that feature rearrangements at chromosomal band 11q23. Similar rearrangements are seen in infant leukemias and are associated with gestational diets that are high in naturally occurring topoisomerase II–active compounds. Finally, regimens that include mitoxantrone and epirubicin are linked to acute promyelocytic leukemias that feature t(15;17) rearrangements. The first part of this article will focus on type II topoisomerases and describe the mechanism of enzyme and drug action. The second part will discuss how topoisomerase II poisons trigger chromosomal breaks that lead to leukemia and potential approaches for dissociating the actions of drugs from their leukemogenic potential.

Keywords

topoisomerase II poison; anticancer drug; chromosomal translocation; acute myeloid leukemia; acute promyelocytic leukemia

Type II topoisomerases are ubiquitous enzymes that regulate levels of DNA under- and overwinding and remove knots and tangles from the genetic material.^{1–6} These enzymes are essential for cell survival and play vital roles in virtually every nucleic acid process, including DNA replication, transcription, and recombination. They also are required for proper chromosome organization and segregation.^{1–6}

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Conflicts of interest

C.A.F. has intellectual property in tools for the molecular cloning of translocation breakpoints, CYP3A4 genotyping, and analyzing topoisomerase II cleavage complexes. The other authors claim no conflicts of interest.

Beyond their critical physiological functions, type II topoisomerases are the targets for some of the most widely prescribed anticancer drugs in clinical use.^{1,2,7-9} However, these enzymes also appear to trigger chromosomal translocations that initiate specific forms of leukemia.^{1,2,10-15}

This article will discuss the role played by type II enzymes in generating chromosomal breaks and the types of leukemias that are associated with different topoisomerase-active agents. As a prelude to these discussions, the article will describe the actions of type II topoisomerases and the mechanisms by which anticancer drugs and natural products convert these essential enzymes into toxic proteins that fragment the genome.

Type II topoisomerases

Humans express two isoforms of type II topoisomerases, A and B.^{1-6,16} These isoforms share extensive amino acid sequence identity (~ 70%) but are encoded by separate genes (located at chromosomal bands 17q21-22 and 3p24 in humans, respectively). Both isoforms are homodimers and display nearly identical enzymological properties, except that topoisomerase II α can distinguish the handedness of DNA supercoils during relaxation reactions.^{1,2,17,18}

Despite their similarities, topoisomerase II α and II β have distinct patterns of expression and separate nuclear functions.^{1-6,16} Topoisomerase II α is essential for the survival of proliferating cells and its expression is linked to cellular growth. It is almost non-existent in quiescent and differentiated tissues, but rapidly proliferating cells contain ~ 500,000 copies of the enzyme. Topoisomerase II α is associated with replication forks and remains tightly bound to chromosomes during mitosis.^{1-6,16,19,20} It is the isoform that functions in growth-related cellular processes and is required for chromosome segregation.

In contrast, topoisomerase II β is dispensable at the cellular level (although it is required for proper neural development in mammals), and its presence cannot compensate for the loss of topoisomerase II α in human cells.^{1-6,16,21,22} In addition, the concentration of topoisomerase II β is independent of proliferation status, and high levels of this isoform are found in most cell types.^{1-6,16,19} Topoisomerase II β dissociates from chromosomes during mitosis but appears to play an important role in the transcription of hormonally and developmentally regulated genes.^{5,20,23,24}

Because of the mechanistic similarities of topoisomerase II α and II β , these enzymes will be referred to collectively as topoisomerase II unless a distinction between the isoforms is made.

Topoisomerase II alters the topological properties of DNA (*i.e.*, supercoiling, knotting, and tangling) by introducing transient double-stranded breaks into the genetic material, transporting an intact double helix (T-segment) through the cleaved DNA “gate” (G-segment), and resealing the original break.^{1,2,4,6,25} The enzyme requires a divalent cation, likely Mg²⁺ *in vivo*, to carry out the necessary nucleic acid chemistry (DNA bending, cleavage, and ligation) and ATP to drive the conformational changes necessary for double-stranded DNA passage.²⁶⁻³⁰ In order to maintain genomic integrity while the G-segment is cleaved, topoisomerase II covalently attaches to the newly generated 5'-termini through phosphotyrosine bonds.³¹⁻³³ This covalent enzyme-cleaved DNA complex is known as the *cleavage complex*.

The homodimeric structure of topoisomerase II plays two critical roles in the double-stranded DNA passage reaction.^{1,2,4,6,25} First, having two subunits allows the protein to form gates through which the T-segment can enter and exit the enzyme-DNA complex.

Second, it provides the enzyme with two active-site tyrosine residues, allowing it to cleave and covalently attach to both strands of the G-segment.

Type II topoisomerases as cellular toxins

Because type II topoisomerases must generate double-stranded DNA breaks prior to strand passage, they are inherently dangerous proteins. Thus, while necessary for cell viability, these enzymes also have the capacity to fragment the genome.^{1–9} As a result of this “Jekyll–Hyde” persona, levels of cleavage complexes must be maintained in a critical balance (Fig. 1).

Cleavage complexes are requisite intermediates in the strand-passage reaction catalyzed by type II topoisomerases. Thus, a decrease in their concentration generally reflects a decrease in overall catalytic activity. Consequently, if cleavage complexes drop below threshold levels, topoisomerase II is unable to completely disentangle daughter chromosomes following replication, and cells die as a result of mitotic failure.

If levels of cleavage complexes increase, cells also suffer catastrophic physiological effects, but for different reasons.^{1,2,7,34–37} When replication forks, transcription complexes, or other DNA-tracking systems attempt to traverse the covalent topoisomerase II–DNA roadblock, accumulated cleavage intermediates are converted to strand breaks that are no longer tethered by protein-linked bridges. The ensuing damage induces recombination/repair pathways that can trigger mutations, chromosomal translocations, or other aberrations. If the DNA breaks overwhelm the repair process, their presence can initiate cell-death pathways. However, if cells recover sufficiently, they may survive but contain damaged chromosomes. In some cases, chromosome aberrations initiate a leukemogenic transformation.^{1,2,10–15}

Topoisomerase II poisons

Chemicals that increase levels of topoisomerase II–DNA cleavage complexes convert the enzyme to a potent cellular toxin that generates the chromosomal damage described above. These compounds are called topoisomerase II poisons to distinguish them from catalytic inhibitors of the enzyme.^{1,2,7–9} Topoisomerase II poisons kill cells by a gain of function, inducing the enzyme to generate DNA strand breaks, as opposed to robbing the cell of the essential functions of the enzyme.

Based on their mechanism of action, topoisomerase II poisons can be categorized into two distinct classes.^{1,2,8,9,38} *Interfacial poisons* bind non-covalently to the cleavage complex at the protein–DNA interface. They intercalate into the double helix at the cleaved scissile bond and impede the ability of topoisomerase II to rejoin the DNA ends.^{8,9,39} In essence, interfacial poisons act as molecular doorstops and prevent the DNA gate from being closed. Examples, including etoposide, doxorubicin, mitoxantrone, and bioflavonoids such as genistein, are shown in Figure 2.

Covalent poisons function distal to the active site of topoisomerase II.^{1,2,38} They contain reactive groups such as quinones or maleimides and covalently adduct to cysteine (and potentially other amino acid) residues.^{40–45} It is believed that covalent poisons increase levels of enzyme-mediated DNA cleavage by altering the conformation of the topoisomerase II N-terminal protein gate. Examples, including epigallocatechin gallate (EGCG), which is prevalent in green tea, and curcumin, which is the major flavor and aromatic component in turmeric, are shown in Figure 2.

Topoisomerase II poisons represent some of the most successful and widely prescribed anticancer drugs worldwide.^{1,2,7–9,38} At the present time, six of these agents are approved

for use in the United States. Topoisomerase II–targeted drugs encompass a diverse group of natural and synthetic compounds and are used to treat a variety of human malignancies.^{1,2,7–9,38} For example, etoposide and doxorubicin (and its derivatives) are frontline therapies for a myriad of systemic cancers and solid tumors, including leukemias, lymphomas, sarcomas, breast cancers, lung cancers, neuroblastoma, and germ-cell malignancies. Furthermore, mitoxantrone is used to treat breast cancer, acute myeloid leukemia, and non-Hodgkin lymphoma. In addition, it is used as a single agent to treat multiple sclerosis.⁴⁶

All clinically relevant topoisomerase II–targeted anticancer drugs affect the activities of both enzyme isoforms. However, the degree to which topoisomerase II α and II β are targeted by any given drug and the relative contributions of the two isoforms to drug efficacy are not well understood.^{1,2,7–9,38} Although some drugs exert stronger effects on one isoform over the other,^{47–50} no truly topoisomerase II α – or topoisomerase II β –specific drugs are available for clinical use at the present time.

The above notwithstanding, there do appear to be isoform-specific ramifications of topoisomerase II–targeted drugs that are relevant to their clinical use. For example, cardiotoxicity is the dose-limiting toxicity of doxorubicin and other anthracyclines, as well as mitoxantrone.^{51,52} Although it was long thought that redox cycling and reactive oxygen species generated by these drugs were responsible for the cardiotoxicity,^{51–53} this hypothesis has been tempered by studies in which toxicity persisted in the presence of reactive oxygen species scavengers.^{54,55} A recent study demonstrated that the cardiomyocyte-specific deletion of topoisomerase II β protected mouse hearts from doxorubicin-induced DNA and mitochondrial damage. (Note that the expression of topoisomerase II α , but not topoisomerase II β , is proliferation dependent. Consequently, differentiated tissues almost exclusively express the β isoform.^{1–6,16,56}) Ultimately, the precise mechanism by which doxorubicin and other drugs induce cardiac damage remains controversial. However, the above makes it likely that cardiotoxicity results, at least in part, from the actions of the drug against topoisomerase II β .^{57,58} In addition, mounting evidence suggests that topoisomerase II β is the isoform primarily responsible for initiating at least some topoisomerase II–associated secondary malignancies (discussed below).^{13,24,57}

Topoisomerase II and leukemia

Although type II topoisomerases are the targets for several important anticancer drugs, these enzymes also have been linked to the generation of specific leukemias.^{10–13} This article will focus on malignancies that feature rearrangements in the *MLL* (mixed lineage leukemia) or *PML* (promyelocytic leukemia) genes, as these are the cancers most closely associated with the type II enzymes.^{1,2,10–13} Topoisomerase II–targeted anticancer drugs, as well as dietary topoisomerase II poisons, have been implicated in the leukemogenic process.

Therapy-related acute myeloid leukemia (t-AML)

The first therapy-related leukemia ascribed to topoisomerase II–targeted drugs was an acute myeloid leukemia (AML) that featured balanced translocations involving chromosomal band 11q23 (Fig. 3).^{59,60} Translocations generally are observed in a 1-kb breakpoint cluster region (BCR) of the *MLL* gene, as compared to the larger 8.3 kb BCR that is associated with *de novo* adult AMLs.^{10,13,14,61} The *MLL* gene encodes a complex multidomain transcriptional regulatory protein that is involved in the epigenetic regulation of hematopoietic and non-hematopoietic targets.^{62–66} The *Hox* genes, which are involved in growth and segmentation, are among the genes that are regulated by *MLL*.^{62–66} *MLL* translocation partners include a wide variety of genes (> 80 have been described), but *AF4*

and *AF9* are observed most frequently.^{24,65,67,68} The C-terminal SET domain of MLL (which contains the histone H3K4 methyltransferase activity of the enzyme) is no longer present in the oncoprotein fusion product of the 5'-MLL-partner-3' rearrangement. A hallmark feature of MLL leukemias is increased expression of the *Hox* genes.^{63–66}

The above t-AMLs were first reported in the 1980s and correlated with the introduction of the epipodophyllotoxins, etoposide, and teniposide into the clinic.^{59,60} There is now a well-established correlation between the use of etoposide and doxorubicin in chemotherapeutic regimens and the development of t-AMLs that include 11q23 rearrangements.^{10,14,24,61} These leukemias are characterized by a short latency period (< 2 years between the treatment of the primary malignancy and clinical diagnosis of the secondary disease) and poor prognosis.^{13,69–72} The occurrence of t-AMLs appears to depend on the specific regimen, total dosage, and schedule employed. Initially, as many as ~ 12% of patients treated with etoposide went on to develop t-AMLs.^{73–77} However, once high-risk schedules were identified and eliminated, that number dropped to ~ 2–3%.^{73–76,78}

The association of topoisomerase II poisons with 11q23-related t-AMLs suggests that leukemogenic chromosomal translocations are initiated by topoisomerase II-generated DNA breaks in the BCR rather than the selection of pre-existing translocations in the marrow of cancer patients. This postulate is supported by the fact that t-AML 11q23 rearrangements have not been detected in marrow samples banked before the start of treatment for the initial malignancy.^{79,80} Moreover, all of the breakpoints mapped to date in *MLL* (whether within or outside of the BCR) and partner genes fall within a few base pairs of a drug-induced enzyme-mediated DNA cleavage site.^{24,80–83} Finally, t-AMLs with 11q23 chromosomal translocations originating in the 1-kb BCR are only observed in patients treated with topoisomerase II poisons and are not associated with regimens that exclude topoisomerase II-targeted drugs.^{10,13,76,77,84–86} This is despite the fact that radiation as well as anti-cancer drugs that alkylate, crosslink, or cleave DNA all generate chromosomal breaks and apoptotic pathways.

Although the above studies argue that topoisomerase II-generated strand breaks play a direct role in the initiation of translocations, there are controversial aspects of this model. It has been suggested that topoisomerase II-mediated DNA cleavage plays an indirect role in the translocation process and that chromosomal rearrangements are initiated by apoptotic nucleases that are induced following drug action.^{24,87–92} This latter mechanism requires that apoptotic pathways be aborted (by processes yet to be described), allowing stable translocations.

In support of the apoptotic mechanism (and in contrast to patient findings), translocations involving 11q23 have been induced in cultured cells by agents that trigger apoptosis but do not target topoisomerase II.^{87,92} Given the high density of etoposide-induced cleavage sites in the BCR (at least *in vitro*), many breakpoints (irrespective of how they were generated) tend to be located within reasonable proximity to a topoisomerase II-mediated cut site.

Notwithstanding the above, there is strong evidence for the direct involvement of topoisomerase II-mediated cuts in the generation of therapy-related acute promyelocytic leukemias (discussed below).

Epidemiological data suggest that etoposide metabolites play a role in triggering 11q23-associated t-AMLs. In humans, etoposide can be metabolized by a number of pathways. Conversion of etoposide to a sulfate or a hydroxy acid inactivates the drug.⁹³ Alternatively, E-ring modifications generated by the cytochrome P450 CYP3A4 convert one of the two methoxyl groups to a hydroxyl moiety, changing etoposide to a catechol metabolite (Fig.

2).^{94–96} A polymorphism in the 5'-promoter region of CYP3A4 (i.e., CYP3A4-V), which is believed to decrease the cytochrome P450-mediated production of etoposide catechol, is associated with a lower risk of 11q23-associated t-AMLs.⁹⁷

Etoposide catechol is present in the plasma of patients treated with etoposide.^{75,94–96,98–101} This compound displays activity against human type II topoisomerases that is similar to that of the parent drug.^{81,102,103} The catechol can be further oxidized to a quinone metabolite by redox cycling or by the actions of cellular oxidases (Fig. 2).^{94–96,102–105} The oxidizing environment of hematopoietic cells suggests that these cells may contain high levels of etoposide quinone.^{81,97,106,107}

Initial studies suggested that etoposide quinone also displayed an activity toward type II topoisomerases similar to that of the parent drug.^{81,102,103,108} However, in hindsight, experiments were carried out under conditions that reduced the etoposide quinone back to the catechol. Under conditions that preserve the quinone, etoposide quinone displays considerably higher activity than the parent drug against human type II topoisomerases.¹⁰⁵ Moreover, etoposide quinone poisons topoisomerase II by a different mechanism than etoposide or etoposide catechol. Whereas the latter two drugs function as interfacial poisons, etoposide quinone is a covalent topoisomerase II poison.^{41,43,44,105}

Although the α and β isoforms both contribute to the actions of topoisomerase II-targeted drugs, emerging findings suggest that topoisomerase II β is the enzyme primarily responsible for generating the DNA breaks that initiate 11q23-associated t-AMLs. The initial evidence supporting this hypothesis comes from a mouse skin carcinogenesis model in which the incidence of secondary malignancy was greatly diminished in skin-specific *top2 β* ^{-/-} knockout mice.⁵⁷ The incidence of etoposide-induced melanomas in the skin of 7,12-dimethylbenz[a]anthracene-treated mice was significantly higher in TOP2 β ⁺ than in skin-specific TOP2 β ⁻ mice. Furthermore, topoisomerase II β was found to be required for etoposide-induced DNA sequence rearrangements and double-strand breaks in cellular models.⁵⁷

Translocations require the juxtaposition of chromosomal partners in order for aberrant repair to take place. Recent evidence indicates that genes are expressed in hubs called transcription factories that bring multiple chromosomes into close proximity.^{109,110} Consequently, it has been suggested that transcription may facilitate the generation of leukemogenic chromosomal translocations.^{109,110} To this point, topoisomerase II β appears to play a much greater role in transcription than topoisomerase II α , and the expression of several genes has been linked to the generation of double-stranded breaks by the β isoform.^{13,23,24,111} As discussed earlier, the two most common translocation partners of *MLL* are *AF4* and *AF9*. In a recent study using cultured KG1 (AML cell) and Nalm-6 (B cell precursor leukemia) human lines, the two partner genes appear to utilize the same transcription factory as *MLL*.^{24,67,68} Moreover, as determined by RNA-FISH, nascent *AF4* or *AF9* transcripts each overlapped the site of *MLL* transcription ~ 2–3% of the time.^{24,67,68} Although topoisomerase II α and II β were both present at the *MLL* locus in this study, the majority of *MLL* breaks generated by etoposide treatment were dependent on the β isoform.²⁴ Finally, the genotoxic effects of etoposide appeared to be mediated primarily by topoisomerase II β .²⁴

Infant leukemias

Treatment-related leukemias with *MLL* translocations have a *de novo* counterpart in acute infant leukemias. *MLL* translocations and rearrangements are present in ~ 80% of cases of infant acute lymphoblastic leukemia (ALL) and ~ 80% of cases of AML in infants and young children.^{10,112–116} Chromosomal translocations initiate *in utero*, as first demonstrated

by molecular cloning of identical non-constitutional *MLL* breakpoint junction sequences in leukemias of monozygous twins.^{10,115,117,118} Rearrangements in 11q23 that are associated with infant leukemias often are more complex than those observed in t-AMLs, and breakpoints are distributed more heterogeneously in the 8.3-kb BCR.¹⁰ However, there is a bias toward the telomeric end of the BCR, and many of the chromosomal breakpoints mapped in infant AMLs are associated with stable topoisomerase II-mediated DNA cut sites.^{10,114,115,119}

Epidemiological studies indicate that mothers who consume diets rich in naturally occurring topoisomerase II-active compounds during gestation increase the risk of infant AML ~ 2–3-fold.^{13,120,121} The topoisomerase II poisons most closely associated with infant AMLs are genistein (which is prevalent in soy products) and other bioflavonoids as well as EGCG and associated green tea catechins.^{120–125} Consistent with this observation, treatment of human cells with genistein induces cleavage in the *MLL* gene.¹²⁰ Furthermore, the incidence of infant AMLs in Japan, where the consumption of soy and green tea is routine, is ~ 2–3 times higher than in the United States.¹²⁶

Taken together, the above data provide strong evidence for a role for topoisomerase II in the etiology of infant leukemias.

Therapy-related acute promyelocytic leukemia (t-APL)

One of the most persuasive arguments for the direct involvement of topoisomerase II-mediated DNA cleavage in generating leukemic chromosomal breakpoints comes from recent studies on t(15;17)(q22;q12) acute promyelocytic leukemias (APLs). These leukemias feature balanced translocations between the *PML* gene on chromosome 15 and the retinoic acid receptor α (*RARA*) gene on chromosome 17.^{127–129} The latency period between treatment and the development of t-APL is relatively short (< 3 years).^{12,15,130}

PML has been implicated in a variety of physiological processes including tumor suppression and apoptosis.^{131–134} PML forms protein aggregates termed PML nuclear bodies, and the disruption of these structures is observed in APL as well as other disease processes.^{15,134–137} The product of the *RARA* gene (*RAR α*) is a nuclear receptor for retinoic acid that plays important roles in mediating the transcription of genes involved in granulocytic differentiation.^{136–138} APLs do not result from the loss of function of PML or *RAR α* . Rather, the chimeric PML–*RAR α* is believed to be a double dominant-negative protein (i.e., the chimera acts as a dominant negative protein for both PML and *RAR α*).^{15,138}

APLs are among the most aggressive types of leukemia, and if untreated can cause death in a matter of weeks.^{139,140} However, in the 1980s and 1990s, the introduction of all-trans retinoic acid and arsenic trioxide, respectively, to treatment regimens dramatically improved the clinical outcome.^{12,15,134,141,142} These targeted treatments induce proteasome-mediated degradation of the chimeric PML–*RAR α* protein, which allows cellular differentiation. Currently, the 5-year survival rate for t(15;17) APLs is ~ 74%.^{138,142}

A role for topoisomerase II in triggering t(15;17) t-APLs was first proposed based on the observation that the increased use of mitoxantrone in breast cancer regimens was paralleled by a increased frequency of t-APL in treated patients.^{130,143} Remarkably, chromosome 15 breakpoints in ~ 1/2 of the t-APL cases that follow mitoxantrone exposure map to a hotspot within *PML* intron 6 that is only 8 bp in length (Fig. 4).^{11,12,143} Moreover, this hotspot is centered on a stable topoisomerase II-mediated DNA cleavage site that is induced by mitoxantrone.¹⁴³ Because the scissile bonds cleaved by topoisomerase II on the two strands of the double helix are separated by 4 bp, all of the breakpoints in the hotspot are located

within 2 bp of the actual enzyme-generated cut site.^{31,32} Further evidence for the involvement of topoisomerase II in t(15;17) t-APLs comes from patients treated with epirubicin (another topoisomerase II poison).¹⁴⁴ Chromosomal breakpoints cluster at epirubicin-induced sites of topoisomerase II-mediated DNA cleavage, which are distinct from the mitoxantrone-related hotspot.^{11,12,144}

In addition to its use as an anticancer drug, mitoxantrone is used as an immunosuppressant to treat patients with multiple sclerosis.⁴⁶ Unfortunately, the use of mitoxantrone in multiple sclerosis regimens correlates with the appearance of t(15;17) t-APLs in patient populations. Overall, multiple sclerosis patients account for ~ 16% of t-APLs.^{12,143,145,146} The chromosomal breakpoints mapped in multiple sclerosis patients are identical to those observed in mitoxantrone-treated cancer patients and are centered on the topoisomerase II-mediated cleavage hotspot in the *PML* gene.^{46,143} Once again, this suggests a critical involvement of topoisomerase II in generating the DNA strand breaks that are processed to form chromosomal translocations. Finally, because t(15;17) t-APLs are seen in multiple sclerosis patients who had no previous history of cancer, this finding provides further evidence that topoisomerase II poisons drive the formation of the leukemic translocations rather than selecting for cells with pre-existing chromosomal damage.

Molecular genesis of leukemic translocations initiated by topoisomerase II

All current models for the molecular genesis of leukemic translocations initiated by topoisomerase II are predicated on the assumption that chromosomal breakpoints are derived from drug-induced sites of enzyme-mediated DNA cleavage. They are based on comparisons between *in vitro* topoisomerase II DNA cut sites and chromosomal breakpoints sequenced from patient samples.^{10,143} In these models, topoisomerase II poisons induce two separate DNA breaks, one on *MLL* or *PML* and the other on its respective translocation partner genes. The single-stranded 5'-overhangs generated by topoisomerase II-mediated DNA cleavage¹⁴⁷ are processed by exonucleolytic digestion or provide the basis for template-directed polymerization to generate complementary bases at the fusion point of the two genes.^{10,143} The free ends of the partner genes are subsequently joined by non-homologous end joining (NHEJ).

Recent studies suggest that the alternative NHEJ (aNHEJ) pathway, rather than classical NHEJ, mediates chromosomal translocations.¹⁴⁸ aNHEJ is initiated by PARP1 recognition of double-stranded DNA breaks and competes with the Ku complex of classical NHEJ.¹⁴⁹ In support of a role for aNHEJ, repression or inhibition of PARP1 dramatically decreased chromosomal translocations in cultured human cells that were treated with etoposide.¹⁴⁸

Summary

Although topoisomerase II-targeted drugs are widely prescribed to treat cancers and other diseases, the use of these drugs and the ingestion of dietary topoisomerase II poisons are linked to specific types of acute leukemia. Regrettably, all current evidence indicates that the induction of these leukemias is mechanism-based as opposed to idiosyncratic. Although the incidence of some therapy-related malignancies can be modulated by the regimen that is employed, the association of topoisomerase II poisons with leukemia appears to be inherent to drug action.

However, there is hope that topoisomerase II-targeted drugs or new regimens with improved safety profiles can be developed. Recent studies suggest a preferential role for topoisomerase II β in the generation of leukemic chromosomal breakpoints. Therefore, at least in theory, the development of agents that are specific for the α isoform could lead to a new generation of topoisomerase II-targeted drugs with less leukemogenic potential.

Finally, the newly defined role of PARP1 in the generation of chromosomal translocations offers a potential strategy for reducing the risk of secondary oncogenic translocations by utilizing PARP1 inhibitors in combination with topoisomerase II poisons.

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Figure 1.

Topoisomerase II is an essential but genotoxic enzyme. The balance between enzyme-mediated DNA cleavage (which is required for its physiological functions) and ligation is critical for cell survival. If the level of topoisomerase II-mediated DNA cleavage decreases below threshold levels, cells are not able to untangle daughter chromosomes and ultimately die of mitotic failure (left). If the level of cleavage becomes too high (right), the actions of DNA-tracking systems can convert these transient complexes to permanent double-stranded breaks. The resulting DNA breaks, as well as the inhibition of essential DNA processes, initiate recombination/repair pathways and generate chromosome translocations and other DNA aberrations. If the strand breaks overwhelm the cell, they can trigger cell death. This is the basis for the actions of several widely prescribed anticancer drugs. If cell death does not occur, mutations or chromosomal aberrations may be present in surviving populations. Exposure to topoisomerase II poisons is associated with the formation of specific types of t-AMLs and infant leukemias that involve the *MLL* (mixed lineage leukemia) gene at chromosome band 11q23 and t-APLs that feature t(15:17) chromosomal translocations between the *PML* (promyelocytic leukemia) and *RARA* (retinoic acid receptor α) genes (lower right arrow).

Anticancer Drugs

Dietary Topoisomerase II Poisons

Figure 2.

Structures of selected topoisomerase II poisons. Clinically used anticancer drugs that target topoisomerase II are shown on the left. Dietary topoisomerase II poisons are shown on the right. The catechol and quinone metabolites of etoposide (generated by CYP3A4 and cellular oxidases or redox cycling, respectively) are highlighted in the red box. Epigallocatechin gallate is abbreviated as EGCG.

Figure 3.

Schematic of the *MLL* locus (chromosomal band 11q23) showing the breakpoint cluster region (BCR). Exons 8–14 are indicated by red rectangles. *MLL* breakpoints cluster within an 8-kb region of the gene (green line). Breakpoints observed in topoisomerase II–associated t-AMLs and infant AMLs are more tightly clustered in the ~ 1 kb telomeric end of the BCR (blue line). Adapted from Ref. 24.

Figure 4.

Schematic of the *PML* locus (chromosomal band 15q22) showing the distribution of breakpoints within intron 6 (bcr1 breakpoint region) in t-APL. Exons 6 and 7 are indicated by red rectangles. Arrows denote *PML* translocation breakpoints identified in patients treated with mitoxantrone (black triangles) or epirubicin (green triangles). The asterisk denotes an 8-base pair breakpoint cluster hotspot observed in mitoxantrone-related t-APLs. A separate cluster was associated with epirubicin-related t-APLs. Chromosomal breakpoints are preferential sites of topoisomerase II cleavage induced by the respective drugs. Adapted from Ref. 144.