[haematologica reports]
2006;2(15):30-33

# Translocation Mechanism in Secondary Leukemias Following Topoisomerase II Poisons

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*C.A.F. supported by NIH Grants R01CA77683, R01CA80175, R01CA85469, Leukemia & Lymphoma Society SCOR, a Grant from Pennsylvania Department of Health, Joshua Kahan Foundation, Friends of the Joseph Claffey Fund. Pennsylvania Department of Health disclaims responsibility for any analyses, interpretations or conclusions. N.O. supported by NIH Grants R01GM33944 and R01GM53960.*

More than 15 years after observations of an association between a distinct form of leukemia characterized by balanced chromosomal translocations involving band 11q23 and introduction of epipodophyllotoxins into clinical usage for anti-cancer treatment in the late1980s, the translocation mechanism is still debated. Epipodophyllotoxins and other anticancer drugs that have been linked to leukemias with various balanced translocations as a treatment complication share the property of being topoisomerase II poisons. In the early 1990s the MLL (*ALL-1, HRX, hTRX1*) gene was cloned by several groups at the genomic breakpoint junctions of translocations disrupting chromosome band 11q23. This paper will review evidence in favor of the hypothesis that topoisomerase II cleavage complexes induced by poisons of this enzyme damage DNA directly and lead to translocations in secondary leukemias with *MLL* translocations.

Topoisomerase II changes DNA topology from supercoiled to relaxed states by creating transient 4-base staggered double stranded breaks that enable passing of a separate intact double helix through the cleaved DNA. Covalent phosphodiester bonds are formed between active site tyrosyl residues (one per each subunit of the topoisomerase II homodimer) and the 5' phosphate residue of the base 3' to the cleavage (reviewed in (1)). The covalent enzyme-cleaved DNA complex is referred to as the cleavage complex. Formation of cleavage complexes is essential to normal cellular functions. The cleavage complex also is the target for several cytotoxic anticancer drugs (reviewed in (1)).

Agents targeting the topoisomerase II cleavage complex that have been implicated in leukemia include the epipodophyllotoxins etoposide and teniposide, the anthracyclines daunorubicin, doxorubicin and 4-epi-doxorubicin, the anthracinedione mitoxantrone, and dactinomycin. These agents share the property of being topoisomerase II *poisons*, i.e. they convert topoisomerase II into a potent cellular toxin by increasing the concentration of cleavage complexes, either by decreasing the religation rate or increasing the forward rate of cleavage (reviewed in (1)). Their desired antineoplastic action is initiation of apoptotic pathways as the cellular response to irreversible DNA damage and illegitimate DNA recombination from the increased cleavage complexes. The stabilization of cleavage complexes by anticancer drugs has led to the hypothesis that resultant chromosomal breakage from perturbation of the topoiomerase II cleavage-religation equilibrium can cause untoward DNA recombination in the form of translocations and lead to leukemogenesis. The action of topoisomerase II poisons is distinct from the action of catalytic inhibitors of the enzyme; however, certain agents such as anthracyclines and dactinomycin behave as topoisomerase II poisons at low concentrations but as catalytic inhibitors at higher concentrations. In the clinical setting topoisomerase II targeted drugs collectively have been called *topoisomerase II inhibitors* (reviewed in (1)).

The balanced translocations in secondary leukemias are many and include diverse translocations of the *MLL* gene at chromosome band 11q23, t(8;21) and variant *AML1(CBFA2)* translocations, inversions and translocations fusing CBFB and MYH11 at chromosome bands 16p13 and 16q22, t(15;17) fusing *PML* and *RARA*, t(8;16) fusing *MOZ* and *CBP*, t(9;22) fusing *BCR* and *ABL*, and diverse *NUP98* translocations involving chromosome band 11p15.[1,2] *MLL*

translocations are the most common translocations in leukemias associated with topoisomerase II poisons. *MLL* translocations generally fuse introns of the breakpoint cluster region (bcr) of *MLL* and one of >50 different partner genes, resulting in a leukemogenic chimeric oncoprotein from the in-frame *5'-MLL-partner gene-3'* rearrangement.[3] The reciprocal 5'-*partner gene-MLL-3'* rearrangement and/or fusion transcript may be present or absent. Secondary leukemias with MLL translocations present clinically as heterogeneous forms of AML, ALL or MDS. There is at least partial overlap with the diverse partner genes found in de novo leukemias with *MLL* translocations (reviewed in (4, 5)).

The genomic breakpoint junction sequences in leukemias in patients and their temporal origins relative to chemotherapy administration are integral to the model implicating topoisomerase II mediated damage in the *MLL* translocation mechanism. The absence of *MLL* translocations in the bone marrow at primary cancer diagnosis by PCR and emergence during the treatment have been consistent findings in pediatric cases of secondary AML following primary neuroblastoma[6,7] or primary ALL.[8] In certain instances, the MLL translocation may not be associated with a leukemia phenotype within the typical latency period from chemotherapy exposure to emergence of disease.[9] Although MLL translocations can be present early at low cumulative doses of topoisomerase II poisons[6] or arise later during treatment,[7-9] tracing of the translocations in sequential marrow samples to timepoints during treatment has suggested that treatment causes translocations rather than selecting for pre-existing clones.

Molecular cloning of *MLL* genomic translocation breakpoint junctions in secondary leukemias has demonstrated heterogeneity in breakpoint distribution and an intron 8 translocation breakpoint hotspot region 3' in the bcr.[6,10-18] The sequences in cases where both genomic breakpoint junctions have been characterized are generally consistent with precise or near-precise interchromosomal recombinations with gains or losses of no or, more commonly, a few bases,[6,10-14] indicating that the translocation breakpoints are in close proximity to the sites of damage. However, complex rearrangements can result, e.g. when MLL and certain partner genes have opposite 5'-3' orientations relative to the respective centromeres of the chromosomes on which they are found.[18,19] *MLL* tandem duplications also may occur.[16] Another consistent feature at the translocation breakpoint junctions has been the short sequence homologies in *MLL* and its partner genes suggesting DNA damage resolution by the DNA double strand break repair mechanism of nonhomologous end-joining (NHEJ).[6, 10-12, 14] NHEJ repair usually introduces small end modifications to create homologous overhangs at DNA double strand breaks in order to join the ends, but accurately joins the unmodified ends of DNA double strand breaks less often.[20] In an exemplary case of secondary ALL, both breakpoint junctions formed by near-precise recombination of more 5' MLL translocation breakpoints with the net gain of a single templated base addition.[11] Near-precise recombinations have occurred in other cases with loss of several bases.[6,12] In a case of secondary AML with t(9;11), which involved the *MLL* translocation breakpoint hotspot, precise recombination occurred between homologous 5'-TATTA-3' sequences without gain or loss of bases.[14]

Epipodophyllotoxins and other topoisomerase II poisons associated with leukemia as a treatment complication[4,5] have been utilized in *in vitro* and cellular model systems seeking to link topoisomerase II mediated damage to the *MLL* translocation mechanism. In addition, CYP3A4 converts epipodophyllotoxin to a catechol metabolite that is readily oxidized to a quinone. Not only are these metabolites topoisomerase II poisons,[21] but redox cycling between them produces ROS,[22] DNA damage from which can stabilize cleavage complexes.[23] Therefore, the association of CYP3A4 genotype with secondary leukemias uncovered by molecular epidemiologic studies[24,25] has formed the basis to investigate the induction of cleavage complexes by etoposide metabolites.

Studies of etoposide metabolites are of further interest because etoposide metabolism to etoposide catechol increases in patients during conventional, multiple-day, bolus etoposide infusions.[26] However, CYP3A5 may also be involved in etoposide metabolism.[27] In addition, recent studies have revealed that a major etoposide-glutathione conjugate is derived from etoposide quinone.[28] Glutathione conjugation of etoposide quinone can protect the DNA from oxidative damage, and there are many glutathione-S-transferase (GST) polymorphisms.[28] Therefore, additional studies are required determine the significance of etoposide metabolites as genotoxins in the translocation process.

Topoisomerase II *in vitro* cleavage assays, which trap and locate cleavage complexes in naked DNA substrates at the sequence level, were the first approach to study the potential causal rela-

tionship between direct DNA damage from drug-enhanced cleavage complexes and the translocations.[11,14,21,29] Topoisomerase II in vitro cleavage assays performed with etoposide or etoposide metabolites demonstrated enhanced cleavage at the *MLL* translocation breakpoint hotspot region and at multiple other sites in the *MLL* bcr and in partner genes of *MLL* depending on the agent.[11,14,21] In addition, doxorubicin exhibited concentration dependent mixed effects of a poison and catalytic inhibitor of topoisomerase II cleavage near certain translocation breakpoints.[30] These assays have suggested a distribution of topoisomerase II cleavage sites induced by anticancer drugs consistent with the translocation breakpoint distribution in secondary leukemias in patients. Observations from molecular cloning of *MLL* translocation breakpoint junctions in leukemias in patients and *in vitro* cleavage assays have led to models in which specific *MLL* translocations could arise via resolution of 4-base, staggered double-strand breaks from drug-induced cleavage complexes at or in the vicinity of the breakpoints by either imprecise NHEJ repair after minimal processing (e.g. exonucleolytic digestion, small insertion) or precise NHEJ repair of homologous overhangs.[11,14,30]

The biochemistry of topoisomerase II interactions with anticancer drugs is pertinent to these models since various anticancer drugs may be associated single strand-specific nicks. For example, each etoposide molecule stabilizes a separate strand-specific nick at either topoisomerase II subunit-DNA bond, such that two etposide molecules are necessary for a DNA double strand break.[31] This *two-drug* model of double occupancy by etoposide for generation of DNA double strand breaks[31] has implications that anticancer agents may stimulate topoisomerase II single strand nicks rather than double strand breaks at/proximal to the translocation breakpoints. However, replication fork collision can generate a complete double strand break at a cleavage complex with a single strand nick.[32]

Cellular model systems seeking to link topoisomerase II mediated damage to the *MLL* translocation mechanism are also in development. In the *in vivo* complex of enzyme (ICE) bioassay, total cellular DNA is isolated from a treated cell population and DNA covalently bound to topoisomerase II is detected with an anti-topoisomerase II antibody by immunoblotting.[14] ICE bioassays in which etoposide and its catechol and quinone metabolites stimulated cleavage complexes in hematopoietic cell lines have corroborated the *in vitro* assays.[14] Recently it also was observed that etoposide treatment of cultured $CD34^+$ cells caused a spectrum of stable *MLL* rearrangements with breakpoint junction sequences suggesting NHEJ repair similar to leukemias in etoposide-exposed patients.[33]

The findings summarized herein are in keeping with the hypothesis that poisoning effects of drug-stabilized topoisomerase II cleavage complexes at or proximal to translocation breakpoints is the damage mechanism in secondary leukemias with *MLL* translocations. Nonetheless, further study is still needed to resolve this question.

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