Cell Cycle 11:17, 3143-3144; September 1, 2012; © 2012 Landes Bioscience

Do transcription factories and TOP2B provide a recipe for chromosome translocations in therapy-related leukemia?

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Chromosomal translocations are a key early event in many leukemias. Translocations involving the mixed lineage leukemia (*MLL*) locus, for example, are observed in a high proportion of neonatal acute lymphoblastic leukemia (ALL), and in therapy-related secondary acute leukemia (t-AL). Therapy related leukemia arises following various combinations of chemo- and radio-therapy, used in the treatment of a primary cancer. It is a very serious problem and incidences are increasing due to better survival rates following primary malignancies and the use of more intensive chemotherapy regimens.

Two classes of cytotoxic anticancer agents are associated with t-AL; these are TOP2 poisons, including etoposide, epirubicin, mitoxantrone, anthracyclines and alkylating agents. Both types of agents induce DNA damage leading to tumor cell death. Alkylating agents form inter-strand crosslinks and other DNA adducts, while TOP2 poisons inhibit relegation of the normally transient, enzyme-bridged DNA break, generated in the reaction cycle of DNA topoisomerase II (TOP2), leading to the accumulation of DNA double-strand breaks (DSBs).[1,2] The subsequent development of t-AL presumably reflects genetic damage induced in hematopoietic stem or progenitor cells by these agents. TOP2 poison-associated t-AL generally appears within a year of exposure to the agent, and karyotypic abnormalities are typically balanced chromosome translocations that generate novel fusion, most frequently involving the *MLL* locus at 11q23, and a number of partner genes including *AF9* and *AF4*.

How recurrent translocations occur in de novo leukemia and in t-AL, and why the same translocations are seen repeatedly, have puzzled clinicians and scientists for decades. We proposed a model[3] for translocations in t-AL, which draws on two aspects of the way transcription occurs in the cell. First, transcription occurs at dynamic structures called factories, consisting of multiple active RNA polymerase complexes each engaged with a separate transcription unit (gene).[4] Second, one of the two TOP2 paralogues, TOP2B appears to have a role in transcriptional activation that involves a transient DSB.[5-7]

In this model, transcription-associated DSBs introduced by TOP2B are stabilized by a topoisomerase poison such as etoposide. The possibility then arises for generation of a chromosome translocation by erroneous repair of heterologous ends, a situation that is facilitated by the close proximity of genes in a common transcription factory (**Fig. 1**).

In support of this hypothesis, the sharing of transcription factories between genes on the same and different chromosomes has been demonstrated previously by high-resolution FISH.[8] In the case of the *MLL* locus, translocation breakpoints found in most (11q23) t-AL cases map to a small region of less than 1 Kb. Notably, this region has the properties of a cryptic promoter,[9] and this and other breakpoint regions contain DNaseI hypersensitive regions indicative of an "open" chromatin structure.

Several testable predictions arise from this model. Taking the example of *MLL* and its translocation partners, *AF9* and *AF4*, actively transcribing *MLL* loci should be present in the same transcription factories as *AF9* or *AF4* in at least some nuclei, and this would be expected to be the case more frequently than for genes that are not *MLL* translocation partners. Overlapping nascent RNA signals detected by RNA FISH confirmed this to be the case for *MLL* and *AF9*, and for *MLL* and *AF4*.[3] Second, etoposide-induced rearrangements induced in the *MLL* locus should be preferentially dependent on TOP2B (as opposed to its paralogue TOP2A). Using DNA-FISH with a diagnostic *MLL* break-apart probe, we showed that the frequency with which *MLL* rearrangements were induced by etoposide was approximately seven-fold lower in a TOP2B null-lymphoblastoid cell line than in its wild-type counterpart.[3] We also noticed that while TOP2B status did not affect the overall rate of DSB induction by etoposide and had only a small effect on cytotoxicity,[10] TOP2B was required for the genotoxic effects of etoposide as assessed by micronucleus formation.[3]

The possible link between the role of TOP2B in transcription and the requirement for TOP2B for the genotoxic effects of etoposide is an area for further study that should yield a better understanding of the mechanism of chromosomal translocations in acute leukemia. Furthermore, the specific role of TOP2B in mediating rearrangements at the *MLL* locus, and overall TOP2 poison-mediated genotoxicity, suggests that TOP2A-specific agents may reduce unwanted genotoxic DNA damage while maintaining the anticancer cytotoxic activity.

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Submitted: 07/11/12; Accepted: 07/12/12
http://dx.doi.org/10.4161/cc.21477
Comment on: Cowell IG, et al. Proc Natl Acad Sci USA 2012; 109:8989-94. PMID:22615413; http://dx.doi.org/10.1073/pnas.1204406109.

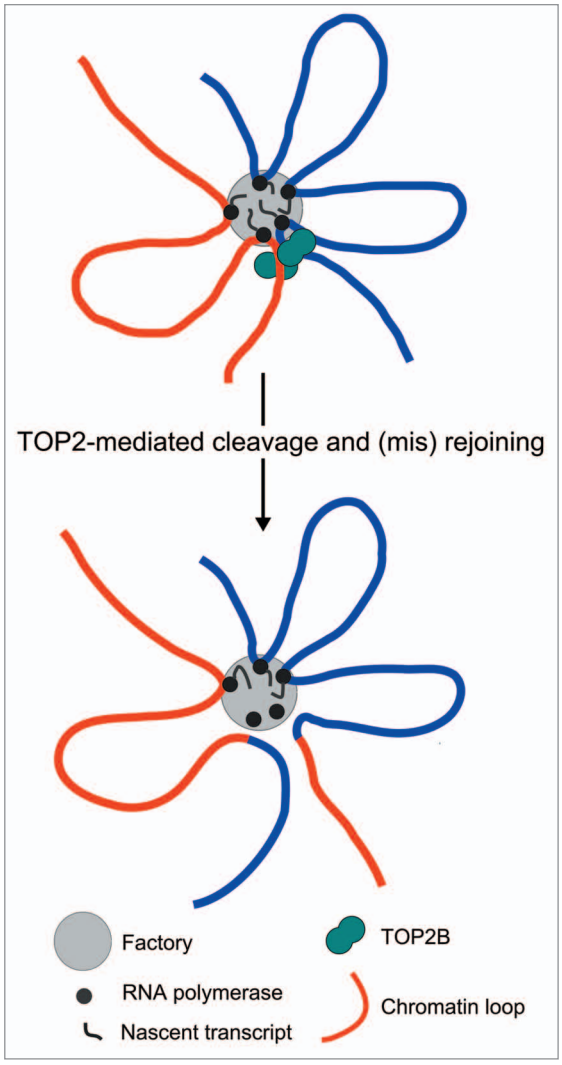

Figure 1. Organization of transcription units into transcription factories and transcription factory model for t-AL-associated chromosome translocations. The focal nature of transcription could facilitate chromosome translocation. Active genes located on different chromosomes (red and blue) can share common transcription factories. Ongoing transcription in a shared transcription factory can maintain two heterologous chromosome segments in juxtaposition for the length of the transcription cycle, and it is hypothesized that this facilitates translocation when DNA breaks are induced. In the case of t-AL, translocations breaks are introduced by the action of TOP2 poisons on TOP2B during transcription. Diagram adapted from Cook et al.[4]

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