トポイソメラーゼ２阻害剤による白血病発症機構の解析

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The goal of this research is to understand how the inhibition topoisomerase II (TOP2) leads to leukemogenesis. TOP2 releases DNA torsions by creating a transient DNA double strand break (DSB). TOP2 inhibitors, like the chemotherapeutic agent etoposide, trap TOP2 in the DNA leading to a permanent DSB that is a source of genome instability. Many cancer patients treated with chemotherapy with TOP2 inhibitors develop a lethal secondary leukemia, a therapy-related Acute Myeloid Leukemia (t-AML), which the driver is a translocation in the gene Mixed-Lineage Leukemia (MLL), that initiates the leukemia in hematopoietic stem cells. TOP2 acts where the DNA folds to form a chromatin loops, including the breakpoint of translocations on MLL. Although DNA folding determines the activity of TOP2 in these locations, transcription accelerates the processing of etoposide induced breaks and facilitates translocations. In this research I explore if preventing loop formation and transcription in hematopoietic stem cells can reduce the fragility of MLL and its translocation upon TOP2 inhibition.

Therapy-related acute myeloid leukemia (t-AML) is a frequent and lethal secondary cancer following chemotherapy and/or radiation therapy. 1.87% of patients treated with chemotherapy develop t-AML, 4.7 times more than in the general population. Due to the increases in survival of cancer patients is expected higher risk of this fatal complication among cancer survivors in the future. Most of the t-AMLs (70%) are driven by a translocation of the driver gene Mixed-Lineage Leukemia (MLL) fused to a great variety of partner genes (like ENL, AF4, AF9, AF10, ELL), which is a driver also of infant acute leukemias. MLL is a histone methyl transferase essential for normal embryonic hematopoietic stem cell (HSC) development, promoting self-renewal and undifferentiation of HSC. Oncogenic chromosomal translocations in MLL generate a fusion protein that lack multiple negative regulatory domains present in the C-terminal of full-length MLL, this leads to a “always active” oncogenic protein that coffers self-renewal and lack of differentiation in HSC and hematopoietic progenitors and represent the beginning of the leukemia. The translocation breakpoints occur in a narrow interval within the MLL locus, two hotspots between exon 9 and 12, called the breakpoint cluster region (BCR).

Topoisomerase 2 (TOP2) poisons including etoposide, mitoxantrone and doxorubicin are successful and extensively used chemotherapeutic drugs for multiple types of cancers in children and adults, but they are highly associated with the occurrence of t-AML, between 2 to 20% (depending on dose and administration schedule) of the patients develop t-AML, by promoting MLL translocations. Topoisomerase II (TOP2) relieves torsional stress in the DNA, like DNA supercoiling and entanglements, by producing a transient DNA double strand break (DSB) and passing a second DNA double helix through the break. During this process TOP2 covalently binds to the ends of the broken DNA, forming a short-lived intermediate called TOP2 cleavage complex (TOP2cc) (Figure 1A). As part of its normal catalytic cycle, TOP2 religates the ends of the DSBs and dissociates from the DNA without causing any damage. TOP2 poisons trap TOP2 in the cleavage state, inhibiting the ligation and leading to a permanent DSB with a protein-DNA adduct that is a source of genome instability and highly cytotoxic for cancer cells. These permanent protein-DNA adduct requires the removal of TOP2 from the DNA ends and subsequent repair of the DSB. Unfortunately, misrepair of these lesions lead to the formation of oncogenic

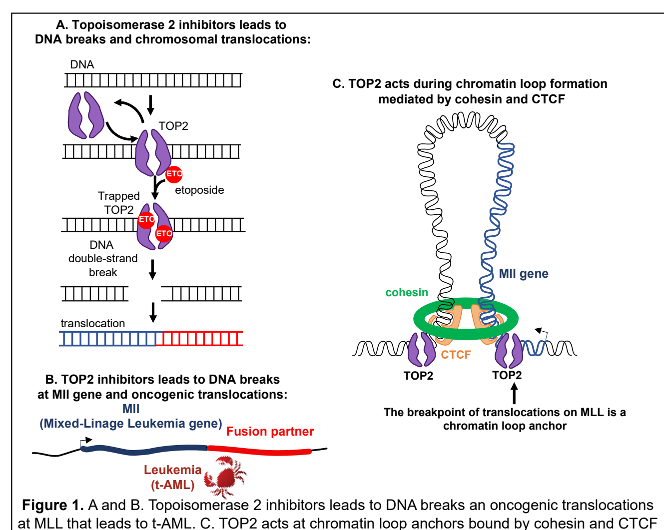

Figure 1. A and B. Topoisomerase 2 inhibitors leads to DNA breaks an oncogenic translocations at MLL that leads to t-AML. C. TOP2 acts at chromatin loop anchors bound by cohesin and CTCF

chromosomal translocations responsible of t-AML (Figure 1B).

In a recent study (Canela A. et al Cell 2017), I used a next generation sequencing method (END-seq) that I developed, to map the location in the genome of TOP2 transient DSBs induced by etoposide (ETO). I found that TOP2 acts where DNA folds to form chromatin loops. Genomic DNA is packed in the nucleus in chromatin loops that regulate DNA replication and transcription. They bring together distant regions in the DNA allowing the interaction between regulatory sequences within the loop. Chromatin loops are formed when ring shaped cohesin complexes translocate along the chromatin until they encounter a pair of CTCF (CCCTC-binding factor) molecules bound to DNA that stop cohesin. This generates a chromatin loop and the base of the loop, referred as loop anchor, is defined by the binding of CTCF and cohesin. I found that TOP2 releases torsional stress in these locations and cohesin binding determines TOP2 breakage, suggesting that the advance of cohesin through the chromatin could generate DNA entanglements or knots that need the activity of TOP2, and will accumulate at loop anchors where cohesin progression is blocked by CTCF. Among these sites of loop anchors were the two hotspots at the breakpoint cluster region of MLL, its fusion partners and other oncogenic drivers (Figure 1C). TOP2 breakage in these locations makes them vulnerable to oncogenic chromosomal translocations in the presence of TOP2 poisons leading to leukemia. In addition, I have found that although cohesin determines the activity of TOP2 and the formation of TOP2cc at loop anchors, transcription accelerates the conversion of these protein adducts into DBSs and facilitates translocations. In conclusion chromosomal translocations induced by TOP2 poisons are determined by loop formation by cohesin and transcription. In this project I propose to target cohesin and transcription to prevent leukemogenic translocations upon the treatment with TOP2 poisons. This will allow future cancer therapies to prevent t-AML during chemotherapy treatment with TOP2 poisons. The initial plan was to assay how loop formation, transcription and nuclear position influence in the ① breakage, ② translocation of MLL upon the treatment of the TOP2 inhibitor etoposide.

1. Map in hematopoietic stem cells (HSCs) TOP2-mediated breakage by END-seq

We used HSC to detect and map the breakage by etoposide by END-seq. We isolated CD34+ stem/progenitor and expanded them in culture for 5 days with cytokines such as Flt3L, SCF, IL-3, IL-6, and TPO, UM729 and we did ChIP-seq for cohesin and CTCF and treated them by etoposide for END-seq (Figure 2A). We detected TOP2 breakage at the two hotspots in the break point cluster region of MLL and overlap with the binding of CTCF and cohesin. And the same for the fusion partners of MLL such as ENL and AF4 (Figure 2B)

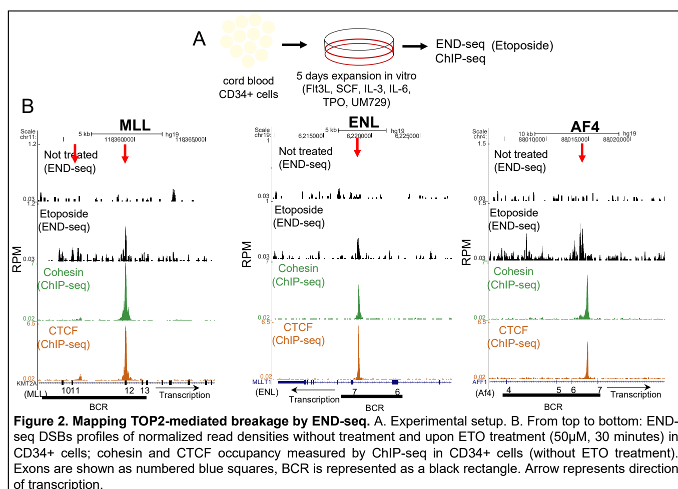

Figure 2. Mapping TOP2-mediated breakage by END-seq. A. Experimental setup. B. From top to bottom: END-seq DSBs profiles of normalized read densities without treatment and upon ETO treatment (50μM, 30 minutes) in CD34+ cells; cohesin and CTCF occupancy measured by ChIP-seq in CD34+ cells (without ETO treatment). Exons are shown as numbered blue squares, BCR is represented as a black rectangle. Arrow represents direction of transcription.

2. Evaluate how loop formation at MLL affects MLL breakage

Next, we assayed how inhibiting or enhancing loop formation affected to the breakage in MLL but we could not do this experiment in hematopoietic stem cells as we could not maintain them long enough in culture to perform gene editing. We used instead mouse Abelson transformed preB cells. We generated Abelson preB cell lines with a degron system induced by auxin (Natsume et al. Cell Rep 2016) to specifically deplete one component of cohesin that is RAD21, and also CTCF (Figure 3A). Depletion of cohesin or CTCF inhibits loop formation. We also targeted the degradation of WALP by auxin induced degron. WALP releases cohesin from the loop anchors. Depletion of WALP increases cohesin in the chromatin and increases loop formation. We performed ChIP-seq for cohesin and CTCF and TOP2 breakage by END-seq with etoposide in these cell lines upon inducing the degron. In WT preB cells we detect TOP2 breakage in the break point cluster region and binding of CTCF and cohesin. In the case of the depletion of CTCF, we do not detect binding of CTCF and cohesin, that does not stop at CTCF sites in absence of CTCF and no breakage by TOP2. If we deplete cohesin (RAD21), we do not

detect cohesin binding, but CTCF is still binding as it is independent of cohesin and the breakage of TOP2 is down to background levels. The opposite situation is in the case of the depletion of WAPL, depletion of WALP increases cohesin binding at the loop anchor sites on MLL and the breakage levels by TOP2 upon etoposide treatment are increased (Figure 3B). The same results were found in the translocation partners of MLL such as AF4. In summary, MLL breakage depends on loop formation and depletion of cohesin or CTCF reduces breakage to basal levels and increased levels of cohesin by depletion of WALP increases MLL breakage.

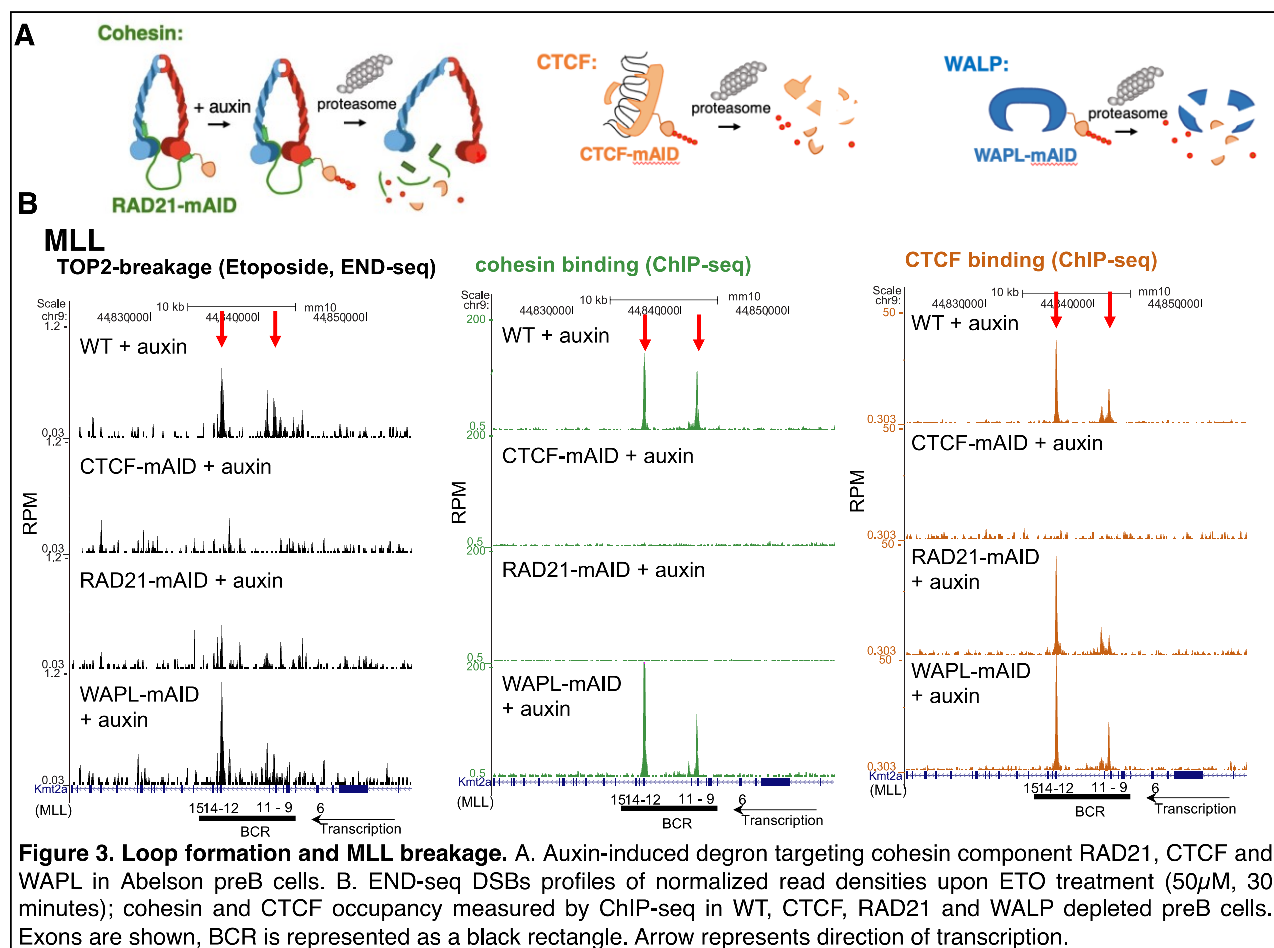

Figure 3. Loop formation and MLL breakage. A. Auxin-induced degron targeting cohesin component RAD21, CTCF and WAPL in Abelson preB cells. B. END-seq DSBs profiles of normalized read densities upon ETO treatment (50μM, 30 minutes); cohesin and CTCF occupancy measured by ChIP-seq in WT, CTCF, RAD21 and WALP depleted preB cells. Exons are shown, BCR is represented as a black rectangle. Arrow represents direction of transcription.

We are now in the process of evaluating how transcription affect to MLL breakage. We constructed in Abelson transformed preB cells an inducible dsRNA targeting specifically of ZFP64, an essential transcription factor for MLL expression. In parallel we are also evaluating how nuclear proximity affects translocation at MLL. We designed gRNA for CRISPR-GO system targeting MLL. CRISPR-GO is an inducible system that reposition genomic loci to the nuclear periphery. Increasing the distance in the nucleus between MLL and its fusion partners would decrease translocations once breakage upon etoposide. We will assay translocation by high throughput translocation sequencing (HTGTS) and FISH.