29ème congrès du CHO
11 au 14 octobre 2023
Giens, Var, France

The dual role of transposable elements in inducing a preleukemic state in HSCs upon TET2 loss and their clonal expansion upon inflammation.

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Clonal hematopoiesis of undetermined potential (CHIP) is defined by the expansion of mutated HSCs with age and **inflammation**. CHIP induces a preleukemic state and an increased risk of developing leukemia. It is mainly driven by mutations in epigenetic factors such as TET2. The molecular mechanisms involved in CHIP remain largely unexplored.

The repressive histone mark H3K9me3 plays a major role in repressing **retroelements (RTEs).** We and others have shown that RTEs, such as LINE-1/L1, are strong inducers of **DNA damage**, **inflammation,** and **transcriptomic alterations** in HSCs. RTEs are thus generally described as oncogenes. However, when their expression rises above a certain threshold, these processes can lead to senescence, apoptosis, and an anti-tumor immune response. Thus, RTEs are also considered tumor suppressors. Their silencing may be required for leukemic progression. TET2 was shown to repress RTE through H3K9me3 in embryonic stem cells. If RTEs are involved in the induction of a preleukemic state and the clonal expansion of *Tet2-/-* HSCs upon inflammation is unknown.

Using H3K9me3 CUT&Tag experiments, we show that a loss of H3K9me3 occurs at the youngest subfamilies of L1, enriched for binding site motifs for transcription factors involved in self-renewal and myeloid differentiation in *Tet2-/-* HSCs. This is associated with a gain of HSC and myeloid signatures in RNA-seq data**.**

We then submitted *Tet2-/-* and WT littermates to a chronic low-dose LPS treatment. Strikingly, while LPS reduces H3K9me3 at L1 in WT, it increases it in *Tet2-/-* HSCs. L1s thus go from a derepressed state in WT to a repressed state in *Tet2-/-* HSCs upon LPS treatment. In vitro, LPS

treatment induces the accumulation of gH2AX foci in WT HSCs, and decreases their viability in single-cell assays, while it has no effect on *Tet2*-/- HSCs. This seems to depend on L1 expression, as WT HSCs are no longer sensitive to LPS after electroporation with L1 shRNAs. Finally, LPS treatment of mice after a competitive repopulation assay induces the loss, in proportion and absolute numbers, of WT but not *Tet2*-/- HSCs in the bone marrow. This suggests that **an H3K9me3-mediated epigenetic switch blocking L1 in *Tet2*-/- HSCs might protect them from L1-induced DNA damage and cell death**.

Altogether, these data suggest that L1s may participate in preleukemic changes induced by Tet2 loss and to the clonal expansion of the mutated HSCs upon chronic inflammation by decreasing the fitness of WT HSCs.