

Investigating the Role of DOT1L in MLL-Mediated Leukemias



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Background

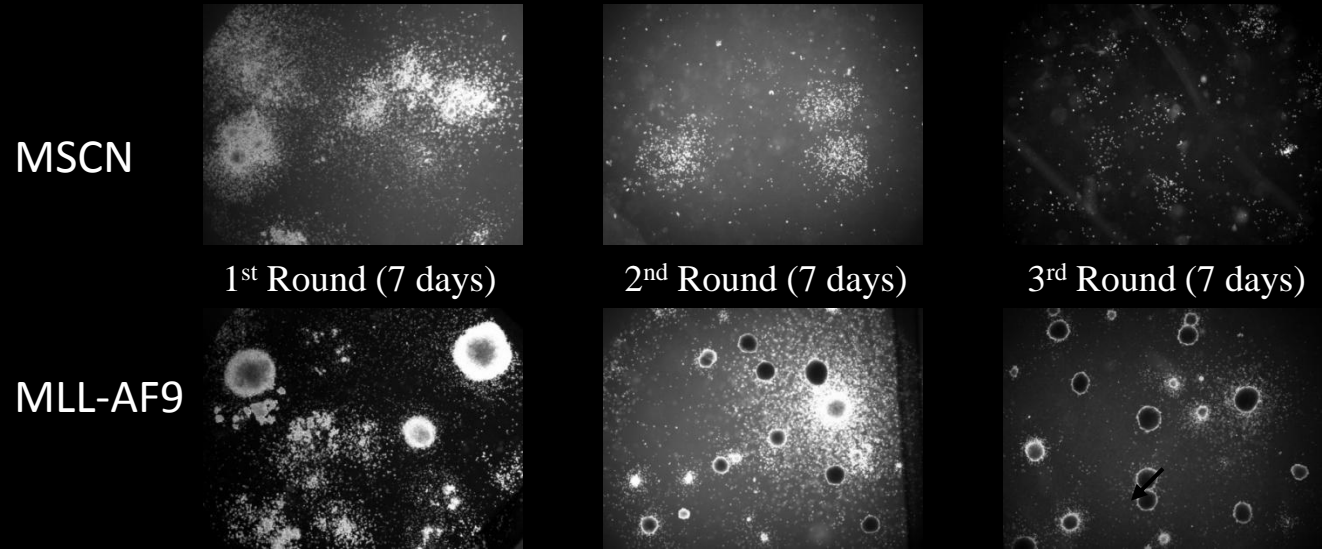
- DOT1L is a histone methyltransferase that catalyzes the transfer of methyl groups to lysine residue 79 of histone H3 (H3K79)
- Histone modifications have been shown to influence gene expression and methylation of the H3K79 mark has been linked to gene activation
- The Zhang Lab has demonstrated that the interaction and enzymatic activity of DOT1L with fusion protein MLL-AF10 is required for leukemic transformation of the mixed-lineage leukemia (MLL) gene
- MLL gene translocations are present in over 70% of infant acute leukemia cases and ~8% of adult acute leukemias.
- While the mechanism of leukemogenesis by MLL fusions is still not precisely understood, at least four more MLL fusion partners (ENL, AF4, AF9, AF17) have the potential to interact with DOT1L
- This raises the possibility that mistargeting of DOT1L may be involved in a universal mechanism responsible for MLL-mediated leukemias

Experimental Design

- To examine the role of DOT1L in MLL-mediated leukemogenesis, an inducible Knock Out (KO) system was created in which hematopoietic progenitor cells (HPCs) could be treated with Tamoxifen to deplete DOT1L
- HPCs from both wild type and mDOT1L heterozygote mice are purified and transduced with retroviruses expressing different MLL-fusion proteins
- If the MLL oncoprotein is capable of transforming the HPCs into leukemic stem cells, the cells will form colonies using a methylcellulose serial plating assay, if cells are not transformed they will terminally differentiate by the second round of plating
- Transformed cells are then collected and treated with Tamoxifen to knockout remaining DOT1L
- If DOT1L is required for maintaining leukemic transformation, the transformed cells will differentiate and no longer give rise to colonies after DOT1L KO.

Preliminary Data

The figure to the right of the screen shows that the oncoprotein MLL-AF9 can be successfully transformed in wildtype HPCs as witnessed by colony formation using serial methylcellulose plating up to the 3rd round. MSCN was an empty vector control and no tightly bound colonies are seen in the 2nd and 3rd rounds of plating.



The graph to the right demonstrates the efficiency of Tamoxifen treatment on induced recombination of DOT1L. The data shows the relative levels of WT DOT1L mRNA in each cell population with Wt/Wt being set to 1.0. Tamoxifen treatment of the 2lox/1lox cells results in no measurable expression of WT DOT1L which is critical for the experimental design of this project.

