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Blood 142 (2023) 5680–5681

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

602.MYELOID ONCOGENESIS: BASIC

Dissecting Lineage and Cell State-Specific Epigenetic Evolution in Relapsed Acute Myeloid Leukemia

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Introduction:
The prognosis for relapsed Acute Myeloid Leukemia (AML) following initial treatment and remission remains poor, largely due to a limited number of therapies with proven clinical efficacy. Epigenetic states and evolution are known to contribute to the initiation of AML, however, the role of epigenetic evolution in relapse remains understudied, and the effects of lineage-specific epigenetic changes at relapse are unknown. We hypothesized that epigenetic modifications confined to specific lineages or differentiation states may contribute to disease resistance and relapse. To test this, we generated single cell ATACseq data from four AML patients at initial diagnosis and at relapse post-induction chemotherapy. We utilized mapping of AML scATAC-seq to scATAC-derived healthy hematopoietic chromatin states, lineage trajectory analysis, and gene and transcription factor accessibility analysis to identify epigenetic changes occurring at discrete points in different AML differentiation trajectories.
Methods:
Malignant AML cells were purified from paired samples collected from four patients at diagnosis and relapse via FACS and were sequenced using the 10x chromium scATAC platform. ArchR, Monocle3, and custom R scripts were used for all analyses. AML cell scATAC data was mapped to a healthy hematopoietic scATAC reference using Latent Semantic Indexing (LSI) and projection onto a single cell ATAC-seq manifold derived from healthy hematopoietic cell types. Closest healthy cell types were identified for each AML cell, differentiation trajectories were generated using monocole3, and gene and transcription factor accessibility was quantified across the different AML differentiation states and lineages.
Results:
Our analysis showed varying patterns of lineage bias across AMLs. Some samples exhibited changes in differentiation state at relapse, becoming either less differentiated or less myeloid-like. Analysis of differences in TF activity between diagnosis and relapse within myeloid and lymphoid trajectories revealed lineage-specific and differentiation state-specific changes in TF activity. In patient SU360, overall differentiation states did not change, and AML cells at both diagnosis and relapse were primarily myeloid-like with a smaller lymphoid-like population. In this sample, GATA family TFs, which were active in progenitor-like cells at diagnosis, became significantly more active in terminally differentiated AML cells at relapse. No specific chromatin changes were observed in lymphoid-like AML cells at relapse. In patient SU484, where diagnosis cells were myeloid-differentiated and relapse cells were more progenitor-like, GATA and RUNX family TF activity, which was restricted to progenitor cells at diagnosis, showed broad activation in all cells at relapse, aligning with the overall de-differentiation observed in the AML. In SU142, a case with no significant change in overall chromatin or differentiation state at relapse, multiple SP family transcription factors showed significant activation specifically in progenitor-like AML cells at relapse.
Conclusion:
Our study of four distinct AMLs suggests the existence of lineage-specific epigenetic evolution in some relapsed AMLs. We found that epigenetic activation of specific transcription factors, such as GATA and RUNX, was restricted to certain steps of myeloid differentiation, suggesting that epigenetic changes contributing to resistance and relapse are relevant in specific AML lineages and differentiation states. Notably, we found that even in AMLs where overall epigenetic state remains stable, there is activation of specific TFs (e.g. SP1) in progenitor cells at relapse, consistent with literature demonstrating SP family TF relevance in Leukemic Stem Cell (LSC) drug resistance. These observations suggest that epigenetic modifications in less-differentiated AML cells may reflect changes occurring in LSCs, potentially contributing to resistance and relapse. Our study is limited due to a small sample size that precludes broad generalizations, but our findings provide a foundation for further characterization of AML relapse epigenetics. Ultimately, these findings suggest the existence of specific epigenetic changes

at certain points in the AML differentiation hierarchy that contribute to relapse, warranting further exploration of cell state-specific epigenetic evolution.

Disclosures Nuno: *Cartography Bio:* Current Employment. **Majeti:** *Orbital Therapeutics:* Current equity holder in private company; *MyeloGene:* Current equity holder in private company, Current holder of *stock options* in a privately-held company; *Pheast:* Current equity holder in private company; *Gilead:* Patents & Royalties; *858 Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Cullgen:* Membership on an entity's Board of Directors or advisory committees; *Roche:* Membership on an entity's Board of Directors or advisory committees; *TenSixteen Bio:* Membership on an entity's Board of Directors or advisory committees; *Kodikaz Therapeutic Solutions:* Membership on an entity's Board of Directors or advisory committees.

https://doi.org/10.1182/blood-2023-180431