Background: The classical hierarchical model of hematopoiesis has been revised based on single-cell RNA sequencing (scRNAseq) data, which suggest a continuous rather than a step-wise differentiation process. However, while differentiation trajectories can be inferred from scRNAseq data, it is impossible to discern whether discreet lineage commitment decisions occur at specific points along these trajectories. The existence of such commitment points could still be compatible with a step-wise differentiation model and their characterization at the molecular level would be essential to accurately model the hematopoietic hierarchy.
Aims: This study investigates DNA methylation programming in normal hematopoiesis to identify novel molecular commitment marks during hematopoietic differentiation. We hypothesized that whole genome DNA methylation analysis facilitates the identification of such molecular commitment marks due to the progressive nature of how this mark is programmed during differentiation.
Methods: Using tagmentation-based whole-genome bisulfite sequencing, we generated a genome-wide DNA methylation map of murine hematopoiesis. This map encompasses 26 murine hematopoietic cell populations throughout the hematopoietic hierarchy. Per cell population we sequenced at least three independent biological replicates. Differentially methylated regions (DMRs) were identified across all populations using DSS without smoothing. Multi-tier single cell scRNAseq data were generated from murine bone marrow using the 10X Genomics Chromium Single Cell 3' kit.
Results: Across all cell populations studied, we identified 147,232 DMRs. Hierarchical clustering of these DMRs revealed co-regulated epigenetic regions that show progressive DNA methylation programming during hematopoietic differentiation. These dynamically regulated regions can be interpreted as specific DNA methylation programs: pan-hematopoietic-, lineage- and cell type-specific DNA methylation programs. The DNA methylation programs were strongly enriched in hematopoietic transcription factor binding sites as well as in lineage and cell type-specific enhancer programs. To gain further insight into how the DNA methylation programming relates to regulation of gene expression, we generated a comprehensive multi-tier single cell gene expression map of murine bone-marrow cells. Integration of DNA methylome programs with single cell gene expression profiles revealed a strong anti-correlation of promoter DNA methylation dynamics and cell-state specific gene expression patterns. The progressive and lineage-specific nature of DNA methylation programming occurring during hematopoietic differentiation allowed us to infer a phylogenetic tree of the hematopoietic system, which is solely based on DNA methylation dynamics. We demonstrated that previously defined subpopulations of common myeloid progenitors (CMPs) show distinct DNA methylation patterns: CD55+ CMPs showed megakaryocyte/erythrocyte patterns, while CD55- CMPs showed DNA methylation patterns compatible with granulocyte and monocyte differentiation. Furthermore, we showed that the identified DNA methylation programs are conserved across different mouse strains which underlines the broad applicability of this resource.
Summary/Conclusion: We provide a novel view on the regulation of hematopoiesis based on DNA methylation dynamics which complements recent findings from scRNAseq studies. Our work provides a rich resource to investigate DNA methylation in normal as well as in abnormal hematopoiesis across a broad range of conditions and mouse strains.

S137 DNA METHYLATION DYNAMICS IDENTIFY LINEAGE-SPECIFIC REGULATION PATTERNS OF HEMATOPOIETIC DIFFERENTIATION

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