3037 – COMBINED SINGLE-CELL DNA METHYLOME AND TRANSCRIPTOME ANALYSIS IDENTIFIES MOLECULAR STATES OF EARLY LINEAGE COMMITMENT

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5-Methylcytosine is a stable epigenetic modification, whose remodeling at specific CpG residues appears integral to the process of enforcing lineage-restricted gene expression. In our previously generated DNA methylation map of the entire murine hematopoietic system we have observed that DNA methylation programming appears to be both progressive and irreversible during hematopoietic differentiation, suggesting that this modification could be used to unambiguously identify molecular marks of lineage commitment.

To identify DNA methylation patterns relevant to lineage commitment, we applied combined single-cell whole-genome bisulfite and transcriptome sequencing on index-sorted Lin- Sca1+ cKit+ hematopoietic stem and multipotent progenitor cells (HSPCs) and on mature lymphoid cells and monocytes. We used our bulk DNA methylome map of the murine hematopoietic system as a template to identify DNA methylation programs of co-regulated CpGs in data obtained from single cells. This allowed us to overcome the critical limitation that single-cell DNA methylomes typically only represent 1-5% of genome-wide CpGs. We were thus able to recapitulate lineage-specific DNA methylation programs identified in bulk-sorted cell populations. In mature cells, DNA methylation programming was mutually exclusive for a specific lineage, supporting the use of single-cell methylomes to establish discreet points at which lineage commitment occurs. Importantly, lineage-specific CpGs were already identified within individual HSPCs, providing a molecular basis for early lineage-priming. This finding was substantiated by the identification of uniform and lineage-specific molecular states within the HSPC compartment, which might still be compatible with a step-wise differentiation process. Lastly, integrative analysis of combined single-cell methylome, transcriptome and index sorting datasets demonstrated that the identified methylome states of lineage commitment are accompanied by changes in lineage-specific gene expression, while the conventional immunophenotypic definition of HSPCs showed only limited correlation with the molecular states.

Together, our work provides proof-of-principle that a high-resolution reference DNA methylation map facilitates the analysis of single-cell DNA methylome data and gives novel insights into the epigenetic regulation of hematopoietic commitment decisions.