3037 – Combined single-cell DNA methylome and transcriptome analysis identifies molecular sattes of early lineage commitment [Abstract]

Sina Stäble, Stephen Krämer, Mark Hartmann, Maximilian Schönung, Jens Langstein, Ruzhica Bogeska, Melinda Czeh, Julia Knoch, Natasha Anstee, Simon Haas, Abdelrahman Mahmoud, Charles Imbusch, Julius Gräsel, Dieter Weichenhan Weichenhan, Lars Feuerbach, Benedikt Brors, Karsten Rippe, Jan-Philipp Mallm, Frank Rosenbauer, Stefan Fröhling, Christoph Plass, Matthias Schlesner, Michael Milsom, Daniel Lipka

Angaben zur Veröffentlichung / Publication details:

Stäble, Sina, Stephen Krämer, Mark Hartmann, Maximilian Schönung, Jens Langstein, Ruzhica Bogeska, Melinda Czeh, et al. 2020. "3037 – Combined single-cell DNA methylome and transcriptome analysis identifies molecular sattes of early lineage commitment [Abstract]." *Experimental Hematology* 88 (Supplement): S50. https://doi.org/10.1016/j.exphem.2020.09.057.

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

Sina Stäble[1,2], Stephen Krämer[3], Mark Hartmann[1], Maximilian Schönung[1], Jens Langstein[1], Ruzhica Bogeska[2], Melinda Czeh[4], Julia Knoch[5], Natasha Anstee[2], Simon Haas[6], Abdelrahman Mahmoud[7], Charles Imbusch[7], Julius Gräsel[2], Dieter Weichenhan Weichenhan[8], Lars Feuerbach[7], Benedikt Brors[7], Karsten Rippe[9], Jan-Philipp Mallm[10], Frank Rosenbauer[11], Stefan Fröhling[12], Christoph Plass[8], Matthias Schlesner[3], Michael Milsom[5], Daniel Lipka[13]*

[1]Section Translational Cancer Epigenomics, Division of Translational Medical Oncology, German Cancer Research Center (DKFZ) & National Center for Tumor Diseases (NCT), Heidelberg, Heidelberg, Germany; [2]Division of Experimental Hematology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [3]Junior Group Bioinformatics and Omics Data Analytics, German Cancer Research Center (DKFZ), Heidelberg, Germany; [4]Hematopoietic Stem Cell Genetics, University of Oxford, Oxford, United Kingdom, Oxford, United Kingdom; [5]Division of Experimental Hematology, German Cancer Research Center (DKFZ); Heidelberg Institute for Stem Cell Technology and Experimental Medicine gGmbH (HI-STEM), Heidelberg, Germany; [6]Heidelberg Institute for Stem Cell Technology and Experimental Medicine gGmbH (HI-STEM); Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany; [7]Division of Applied Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany; [8]Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), Heidelberg, Germany; [9]Division of Chromatin Networks, German Cancer Research Center (DKFZ), Heidelberg, Germany; [10]Division of Chromatin Networks, German Cancer Research Center (DKFZ); scOpenLab, German Cancer Research Center (DKFZ), Heidelberg, Heidelberg, Germany; [11]Molecular Tumor Biology, University of Muenster, Muenster, Germany, Muenster, Germany; [12]Division of Translational Medical Oncology, German Cancer Research Center (DKFZ) & National Center for Tumor Diseases (NCT), Heidelberg, Heidelberg, Germany; [13]Section Translational Cancer Epigenomics, Division of Translational Medical Oncology, German Cancer Research Center (DKFZ) & National Center for Tumor Diseases (NCT), Heidelberg; Medical Faculty, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, Heidelberg, Germany

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.