The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Early Molecular Steps in Human Bone Marrow Stem Cell Differentiation Trajectories

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Understanding the molecular signatures of human hematopoietic stem/progenitor cells (HSCPs) and gene network changes leading to differentiation induction are of utmost importance to achieve therapeutic hematopoietic stem cell (HSC) expansion. The identification of surface markers has improved the prospective isolation of human bone marrow HSCs over the last decades, but the isolated population still remains very heterogeneous. Single cell sequencing approaches have the potential to unbiased assort stem cells along their differentiation stages and trajectories, and to decipher molecular markers in differentiation hierarchies. However, whole transcriptome single cell sequencing often suffers from inadequate detection of low-expressed genes with increased drop-outs.

To address this limitation and to provide sensitive mRNA quantification in human HSPCs, we developed a selected targeted gene panel (600 genes) in combination with 50 protein markers to cover most relevant genes/proteins of early HSPC differentiation and cell cycle status. We employed single cell CITE-Seq (cellular indexing of transcriptomes and epitopes by sequencing) based multi-omic profiling to >60.000 high-quality FACS-enriched human bone marrow HSPCs (CD34$^+$ and CD34$^+$CD38$^-$ cells) from 15 healthy donors representing young, mid and old age groups.

Here, we demonstrate the most comprehensive molecular map of early human HSC differentiation and provide evidence of early branching points into lineage trajectories across different age groups using Slingshot pseudotime analysis. Our data demonstrate that the HSC branched into two main lineages, with the first branching point giving rise to megakaryocytic and erythroid progenitors (MKP/ERP), followed by lymphoid and myeloid progenitors (LMP/MDP). As expected, the most immature HSCs were defined by high expression of CD34, CD90, *CRHBP, HOPX, HLF, MLLT3*. However, we observed a number of hitherto undescribed HSC markers. The MKP/ERP lineage was defined by *GATA1, TFRC, CD36, MPL* and *VWF* expression. Furthermore, we found CD274, the Programmed Cell Death receptor ligand 1, to be selectively expressed in the MKP cluster. Re-clustering and trajectory analysis of the most immature CD34$^+$CD38$^{-/low}$ cells confirmed early lineage commitment, suggesting that an MKP/ERP development does not include myeloid or lymphoid progenitors. A similar pattern was observed for all age groups. Interestingly, we observed increased expression of novel marker genes (*HLA-E, DLK1, ADGRG6, AD-GRG1, MMRN1*) and proteins (CD137 and CD273) in the most immature HSCs. Besides a gradual upregulation of CD38 along differentiation, differentiated cells showed lower CD45 levels. Furthermore, MKP/ERP committed cells showed a downregulation of *SPINK2*. TradeSeq analysis defined the continuous single cell expression changes in genes and markers upon early differentiation and lineage commitment, allowing the detection of decision-making gene networks.

Our data adds further evidence to the revised model of hematopoiesis that challenges the dogma of a common myeloid progenitor giving rise to erythroid, megakaryocytic and myeloid cells. Furthermore, we see a structured hierarchical organization with a sequential loss of lineage potential and not an early commitment of uni-lineage restricted precursors. We report on the upregulation of *ADGRG6, ADGRG1, DLK1, HLA-E, MMRN1* CD137 and CD273 in the most immature HSCs. Molecular and functional characterization of CD273 hi and low HSCs are presented in an adjacent report by our group at this meeting (Schmachtel et al.). In conclusion, we provide a valuable resource for the continuous regulation of genes and surface proteins along early differentiation of very immature human HSCs.