A Diversity of Human Hematopoietic Differentiation Programs Identified Through In Vivo Tracking of Hematopoiesis in Wiskott-Aldrich Syndrome Patients

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Abstract
Several studies have highlighted murine hematopoietic stem cell (HSC) heterogeneity using single cell transplantation, clonal tracking barcoding analysis as well as RNAseq single cell analysis. Here we have used data from a gene therapy trial to treat Wiskott-Aldrich syndrome (WAS) to explore hematopoiesis in humans. In the trial, the therapeutic vector (lentivirus) integrates into the genome at unique positions in each hematopoietic stem and progenitor cell (HSPCs) and is consequently transmitted to all its progeny. Thus hematopoietic ontogeny in humans can be inferred by tracking the appearance of unique integration sites in fractionated blood cell populations. We concentrated on four WAS patients treated by gene therapy with two distinct sources of autologous HSPC : bone marrow (BM) or mobilized peripheral blood (MPB) (following administration of granulocyte colony-stimulating factor (G-CSF)). In these patients, we have sorted peripheral blood samples for 5 cell types: myeloid (granulocytes and monocytes) and lymphoid subpopulations (T, B and NK cells), and analysed their IS profile (using our new optimized pipeline, INSPIIRED). Each IS corresponds to a particular stem/progenitor cell clone, for which we can quantify its contribution in each of the 5 lineages. Using this approach, we have characterized up to tens of thousands IS per patients, including two timepoints of follow up (1 y and 3 y) in order to study longitudinal dynamic. Statistical methods to account for sparse sampling and imperfect cell purifications comprise an important part of our approach and are under development. In initial analysis, using clustering algorithms, we identified different groups of IS clones corresponding to different human hematopoietic differentiation programs. We showed that a significant fraction of IS clones are detected in a single lineage, while other IS clones are characterized by different levels of contribution to the myeloid and lymphoid lineages, highlighting the heterogeneity of human HSC. Clones contributing to all 5 lineages are readily recovered but this study also unravels a diversity of inferred hematopoietic programs with various potentials contributing to human blood homeostasis. Longitudinal analysis of clonal dynamics is ongoing, with preliminary results showing the maintenance of this heterogeneity of HSPC over time. We will also present the differences of hematopoietic programs observed between the two

sources of HSPCs (BM or MPB). These new findings and approaches suggest the existence of various types of human HSPC and provide unique data on human hematopoiesis.