
The dynamics of hematopoiesis over the human lifespan

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SUPPLEMENTARY NOTES

Supplementary Note 1

Both PBA and StatOT model cell trajectories with the same diffusion-drift equation representing Waddington's landscape, resulting in an estimate of the lineage commitment of each cell, for each lineage (i.e., lineage fate probabilities). While these two methods differ in their underlying mathematical approaches, they produced nearly identical results.

Supplementary Note 2

GEP usage increased lineage-associated gene identification by 6.7% for the erythroid, 9.6% for the granulocyte, 16% for the megakaryocyte, 1.4% for the lymphoid, and 8.8% for the monocyte lineages.

Supplementary Note 3

The pediatric auxiliary lymphoid program contains markers of proliferation and nucleic acid metabolism (*PCNA*, *DUT*, *TYMS*), consistent with expansion of this progenitor compartment during active B-cell differentiation occurring at this age¹. Additional pediatric auxiliary lymphoid program genes were associated with the high-mobility group family of proteins (*HMGB1*, *HMGB2*, *HMG2*), ubiquitin-related pathways (*UBB*, *SUMO2*, *UHRF1*), and tubulin formation (*TUBA1B*, *TUBB*, *STMN1*).

Supplementary Note 4

For both unilineage dominance metrics, we first quantify the usage levels for all six lineage GEPs in single cells and select the highest used GEP as the dominant GEP. The first metric divides the usage level of the dominant GEP by the average usage level of the five non-dominant GEPs to define unilineage bias normalized to overall GEP usage. The second metric subtracts the average usage of the five non-dominant GEPs from the usage level of the dominant GEP to define the overall magnitude of unilineage commitment. In both approaches, a higher score should indicate a higher degree of individual lineage bias/commitment. To determine whether the division- or subtraction-based approach more accurately predicted lineage bias of immunophenotypic HSCs, we aimed to validate these scores using clonal output assays on individual Lineage⁻CD34⁺CD38⁻CD90⁺ cells from fetal, adult, and elderly donors. Following outgrowth of single cell clones in culture conditions supportive of differentiation into five lineages, we quantified absolute cell numbers from each lineage using flow cytometry for lineage-specific surface markers as inputs into unilineage dominance scoring (Extended Data Figure 6D, E).

Supplementary Note 5

Our observation of more fetal-HSC-GEP utilization during midgestation than early gestation was inconsistent with this expression program solely resulting from processing.

Supplementary Note 6

Although CD69⁺ HSCs have not been reported in previous studies, we interrogated six independent human datasets and found that CD69 expression marked fetal-HSC-GEP usage regardless of anatomic location (liver or bone marrow) and processing (Extended Data Figure 7C)²⁻⁷. We additionally found that in independent human datasets this CD69-marked fetal-HSC-GEP was also expressed to a higher degree in fetal HSPCs than early postnatal HSPCs, and was expressed equivalently in both FL and bone marrow (Extended Data Figures 7D-E)^{4,5}. Lastly, we found that this GEP was expressed to a higher degree in fetal HSPCs compared to adult HSPCs in an independent mouse dataset (Extended Data Figure 7F)⁸. These results indicate that this fetal-HSC-GEP is not an artefact unique to our dataset, and that fetal use of this GEP is evolutionarily conserved. We additionally sought to identify mechanisms by which this fetal-HSC-GEP is silenced after birth. To that end, we probed a DNA methylation dataset profiling fetal and postnatal HSCs and found that after birth there is increased DNA methylation at the top 200 genes contributing to the fetal-HSC-GEP (Extended Data Figure 7G)⁹.

Supplementary Note 7

However, a recent study specifically investigating the cell surface proteome of human fetal liver detected CD69 as one of many proteins on the surface of HSPCs when using an alternative tissue processing methodology⁷.

Supplementary Note 8

These parameters included age at diagnosis, bone marrow blast percentage, ELN 2017 criteria, FAB classification, and mutational status of *CEBPA*, *FLT3*-ITD, *IDH*, *NPM1*, *RAS*, or *TP53* (Extended Data Figure 9B)¹⁰. We also did not find a significant difference in composite age score when comparing AMLs preceded by myelodysplastic syndrome (MDS) or not (Extended Data Figure 9C). Interestingly, composite age score did not predict survival independently of *DNMT3A* mutation status, raising the possibility of an association with dysregulated methylation (Extended Data Figure 9B).

Supplementary Note 9

It is unclear whether this latter finding was due to differences in the biology of AMLs profiled by the BeatAML study and the TCGA study, or due to the TCGA AML dataset being powered less than the BeatAML dataset (N=257 in BeatAML, N=151 in TCGA).

Supplementary Note References

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