Lineage bias in hematopoietic stem cells: more niche or intrinsic factors?

Taha Bartu Hayal and Chuanfeng Wu

Translational Stem Cell Biology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

Correspondence: C. Wu
wuc3@mail.nih.gov

Received: September 2, 2025.
Accepted: September 29, 2025.
Early view: October 9, 2025.

https://doi.org/10.3324/haematol.2025.288704

Hematopoiesis is organized as a developmental hierarchy with long-term (LT) hematopoietic stem cells (HSC) at the apex, with these cells possessing both multilineage differentiation potential and long-term self-renewal capacity. Lineage commitment is shaped by a complex interplay of intrinsic and extrinsic factors, and the phenomenon of lineage bias in HSC has been a persistent and sometimes contentious topic in the field. Over the years, numerous models and lineage-tracing approaches have been used to probe this question.[1,2] In this issue of *Haematologica*, Shamie and colleagues revisit the topic using an elegant combination of mitochondrial single-cell ATAC sequencing (mt-scATAC-seq) of human HSC and transplantation experiments in mice.[3] By exploiting naturally occurring somatic mutations in mitochondrial DNA as "genetic barcodes," they tracked clonal relationships in human CD34$^+$ hematopoietic stem and progenitor cells during short-term *ex vivo* differentiation, while parallel murine studies assessed *in vivo* lineage output from highly purified LT-HSC. Their results challenge the notion that strong, pre-determined lineage bias is a dominant feature of the HSC pool, instead revealing a more modest, context-dependent intrinsic influence that is substantially shaped by the hematopoietic niche.

The first part of the study exploited mt-scATAC-seq, a method that simultaneously profiles nuclear chromatin accessibility and sequences mitochondrial DNA at single-cell resolution. Nuclear accessibility profiles inform lineage identification, while somatic mitochondrial variants, which accumulate over time and are stably inherited by daughter cells, serve as endogenous barcodes. Together, this enables concurrent lineage tracing and lineage identification. In CD34$^+$ cells from healthy human donors, the authors identified dozens of clones defined by distinct mitochondrial variants. Clonal tracking over 72 hours of *ex vivo* differentiation culture with cytokine conditions supporting multilineage differentiation revealed that most clones contributed broadly across myeloid and erythroid lineages, with minimal evidence of clonal bias. Quantitative analyses, including entropy-based metrics, detected negligible lineage skewing, and smaller clones with low cell counts accounted for the few instances of apparent bias. These results align with those of prior human gene therapy clonal tracking studies,[4,5] which also observed broadly multipotent output *in vivo*.

The second arm of the study by Shamie and colleagues focused on murine LT-HSC (CD34$^-$ LSK CD150$^+$CD48$^-$ cells) in transplantation models. Single cells or defined small numbers (10 or 100) of LT-HSC were transplanted into lethally irradiated recipients, with donor cells distinguished by CD45 alleles. As expected, single-cell recipients displayed variable myeloid to lymphoid ratios in peripheral blood and bone marrow, a variation that could suggest intrinsic bias. However, when larger numbers of LT-HSC were transplanted, the variability remained strikingly similar, indicating that stochastic or host-dependent effects were not averaged out by increasing donor cell number. This persistence of variability even with larger graft sizes argues against strong, fixed intrinsic bias as the dominant driver of lineage composition. Instead, the findings suggest that post-transplant hematopoiesis in irradiated hosts is shaped substantially by environmental and inflammatory cues, which are known to influence progenitor cell behavior and niche interactions. The findings echo and extend several recent lines of evidence. Mathematical modeling has predicted that apparent myeloid bias may emerge from combined HSC and progenitor cell biases, particularly under inflammatory conditions, rather than being solely programmed at the stem cell level.[6] Fate mapping in unperturbed mice has likewise suggested that HSC expansion potential and lineage output are strongly influenced by niche context.[7] These findings reinforce the view that lineage bias, while evident in certain settings, may not represent the dominant mode of hematopoiesis under steady-state or transplantation conditions. As illustrated in Figure 1, hematopoietic clonal bias arises from a dynamic interplay of intrinsic and extrinsic factors. Intrinsic influences include transcriptional

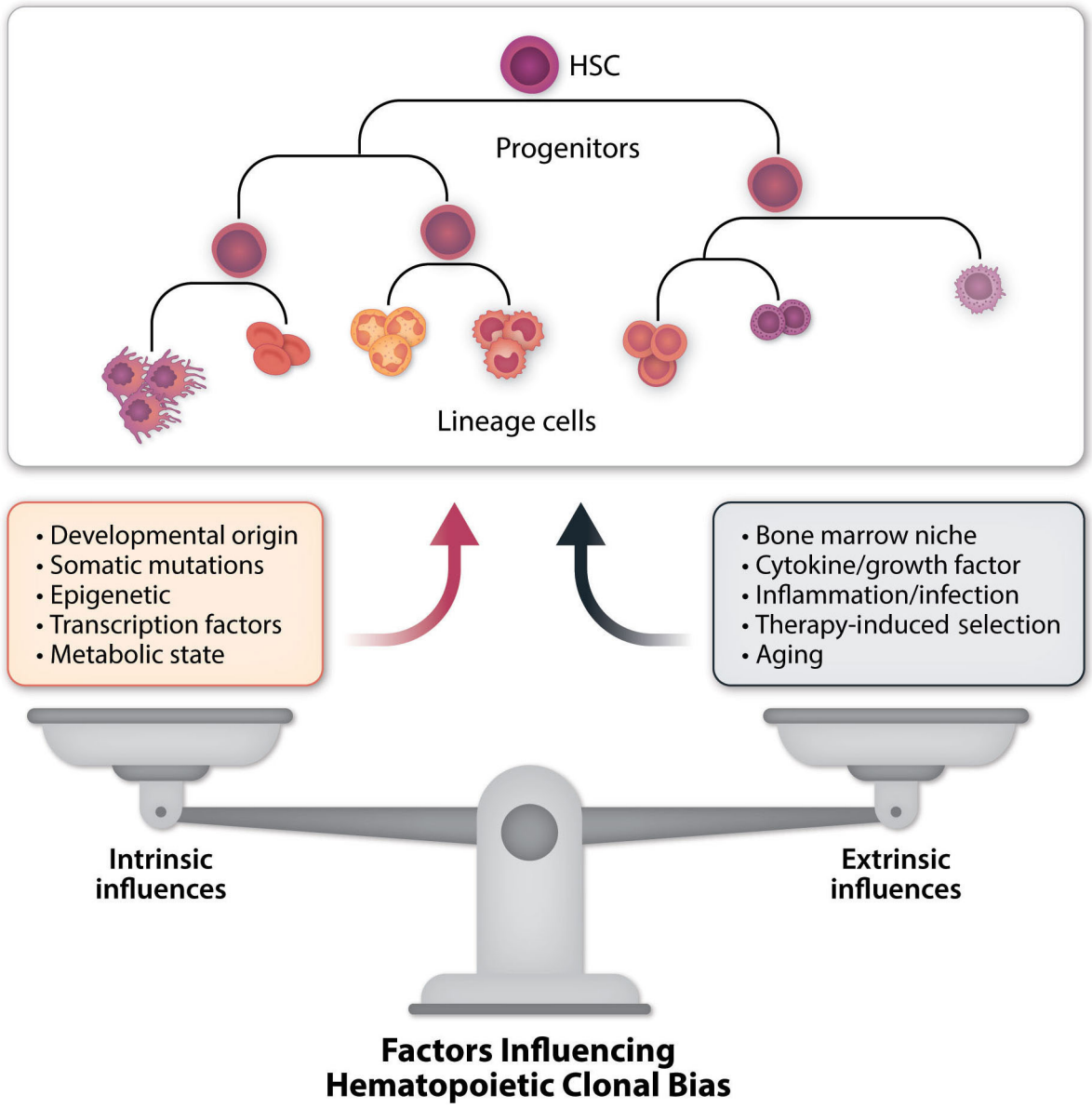

Figure 1. A schematic illustrating the factors that influence hematopoietic clonal bias. Hematopoietic stem cell (HSC) lineage output is shaped by intrinsic factors (shown in the red box) and extrinsic environmental factors (shown in the blue box).

programs, genetic mutations, epigenetic states,[8] and metabolic profiles,[9] and all of these can impact progenitors toward specific lineage fates. Extrinsic factors originate from the bone marrow microenvironment,[10] including cytokines, growth factors, and cell–cell interactions, and are further shaped by systemic factors such as inflammation, infection, therapy, and aging. These findings emphasize the need for careful interpretation of lineage skewing and the value of integrating diverse methods, systems, and timescales to fully capture stem cell behavior for both basic biology and therapeutic translation.

The study by Shamie and colleagues has several strengths. mt-scATAC-seq leverages mitochondrial variants as endogenous barcodes, avoiding confounders of other genetic barcoding methods such as viral integration, fluorescent protein immunogenicity, or stress from gene editing, and, when paired with nuclear accessibility data, enables integrated clonal and lineage analyses. The experimental design combining human *ex vivo* and murine *in vivo* systems allows broader interpretation of lineage bias and niche effects, with murine transplantation extending observations over months to capture long-term repopulation. These experiments were rigorously controlled, with precise LT-HSC purification, infusion cell numbers, and donor and recipient preparation, minimizing technical variability and clarifying biological differences. Limitations include the reliance on human *ex vivo* culture, which may not fully reflect *in vivo* lineage fates, and the use of irradiation in murine transplantation, which perturbs the hematopoietic environment. Longer-term follow up of xenotransplantation of barcoded human HSC or non-toxic conditioning in unmanipulated animals could help to distinguish natural from niche-driven effects. Future studies should investigate how stressors such as aging or inflammation alter the balance between intrinsic and extrinsic influences and integrate mitochondrial barcoding with transcriptomic and epigenomic profiling over time to reveal whether subtle molecular biases precede or accompany shifts in clonal output.

Disclosures

No conflicts of interest to disclose.

Contributions

TBH and CW wrote the manuscript.

References

1. Cordes S, Wu C, Dunbar CE. Clonal tracking of haematopoietic cells: insights and clinical implications. Br J Haematol. 2021;192(5):819-831.
2. Wagner DE, Klein AM. Lineage tracing meets single-cell omics: opportunities and challenges. Nat Rev Genet. 2020;21(7):410-427.
3. Shamie I, Bliss-Moreau M, Lee JC, et al. Comparative single-cell lineage bias in human and murine hematopoietic stem cells. Haematologica. 2026;111(2):632-645.
4. Six E, Guilloux A, Denis A, et al. Clonal tracking in gene therapy patients reveals a diversity of human hematopoietic differentiation programs. Blood. 2020;135(15):1219-1231.
5. Biasco L, Pellin D, Scala S, et al. In vivo tracking of human hematopoiesis reveals patterns of clonal dynamics during early and steady-state reconstitution phases. Cell Stem Cell. 2016;19(1):107-119.
6. Singh A, Chia JJ, Rao DS, Hoffmann A. Population dynamics modeling reveals that myeloid bias involves both HSC differentiation and progenitor proliferation biases. Blood. 2025;145(12):1293-1308.
7. Pinho S, Zhao M. Hematopoietic stem cells and their bone marrow niches. Adv Exp Med Biol. 2023;1442:17-28.
8. Scherer M, Singh I, Braun MM, et al. Clonal tracing with somatic epimutations reveals dynamics of blood ageing. Nature. 2025;643(8071):478-487.
9. Hajishengallis G, Li X, Chavakis T. Immunometabolic control of hematopoiesis. Mol Aspects Med. 2021;77:100923.
10. Man Y, Yao X, Yang T, Wang Y. Hematopoietic stem cell niche during homeostasis, malignancy, and bone marrow transplantation. Front Cell Dev Biol. 2021;9:621214.