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

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Hematopoiesis is organized as a developmental hierarchy with long-term hematopoietic stem cells (LT-HSCs) at the apex, 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 HSCs 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, Shamie and colleagues revisit this assumption using an elegant combination of mitochondrial single-cell ATAC sequencing (mt-scATAC-seq) of human HSCs and transplantation experiments in mice(3). By exploiting naturally occurring somatic mutations in mitochondrial DNA as “genetic barcodes,” they track clonal relationships in human CD34⁺ HSPCs during short-term *ex vivo* differentiation, while parallel murine studies assess *in vivo* lineage output from highly purified LT-HSCs. 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 exploits 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 identify dozens of clones defined by distinct mitochondrial variants. Clonal tracking over 72 hours of ex vivo differentiation culture with cytokine conditions supporting multi-lineage differentiation, revealed that most clones contribute broadly across myeloid and erythroid lineages, with minimal evidence of clonal bias. Quantitative analyses, including entropy-based metrics, detect negligible lineage skewing, and smaller clones with low cell counts account for the few instances of apparent bias. These results align with prior human gene therapy clonal tracking studies (4, 5), which have also observed broadly multipotent output in vivo.

The second arm of the study focuses on murine LT-HSCs (CD34⁻ LSK CD150⁺CD48⁻) in transplantation models. Single cells or defined small numbers (10 or 100) of LT-HSCs are transplanted into lethally irradiated recipients, with donor cells distinguished by CD45 alleles. As expected, single cell recipients display variable myeloid to lymphoid ratios in peripheral blood and bone marrow, a variation that could suggest intrinsic bias. However, when larger numbers of LT-HSCs are transplanted, the variability remains strikingly similar, indicating that stochastic or host dependent effects are 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 programs, genetic mutations, epigenetic states (8), and metabolic profiles (9), and all of which 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.

This study 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 HSCs or non-toxic conditioning in unmanipulated animals could help 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.

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Figure legend :

This schematic illustrates the factors influencing 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).

