



Hematopoietic Stem Cells in Health and Disease—Insights from Single-Cell Multi-omic Approaches

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Abstract

Purpose of Review Hematopoietic stem cells (HSCs) are responsible for the lifelong production of blood and immune cells. This review provides an overview of how single-cell (multi)-omic approaches have recently advanced our understanding of healthy hematopoiesis, hematological malignancies, and the stem cell niche.

Recent Findings Single-cell technologies have revealed tremendous heterogeneity of the HSC compartment, conflicting with the classical view of hematopoiesis. Large-scale single-cell approaches mapping the entire hematopoietic system have enabled an ordering of the observed heterogeneity along meaningful differentiation and cell-state trajectories. These studies provided novel insights into lineage commitment pathways and led to the suggestion of advanced models of hematopoiesis. Single-cell multi-omic technologies, where several entities of individual cells are measured in parallel, have permitted the fine mapping of clonal and developmental differentiation hierarchies, and revealed the molecular consequences of clonal diversification.

Summary Recent single-cell approaches have changed our perception of healthy hematopoiesis, provided an understanding of hematological malignancies at unprecedented depth, and revealed new insights into the stem cell niche.

Keywords Hematopoietic stem cells · Single-cell approaches · Single-cell multi-omics · Hematological malignancies · Leukemia · Stem cell niche

Introduction

The majority of blood and immune cells are constantly replenished by a small population of hematopoietic stem cells (HSCs) located in the bone marrow [1]. Multipotent HSCs give rise to progressively restricted progenitors, finally resulting in the generation of mature blood and immune cells. In the classical model of hematopoiesis, stem and progenitor cell populations have been conceptualized to form discrete cell types resulting in a rigid tree-like model [2, 3]. In this model, HSCs can give rise to additional HSCs in a process

called self-renewal, or produce multipotent progenitors (MPPs) that exhibit reduced self-renewal capacity while maintaining multipotency. Subsequently, MPPs diversify into oligopotent progenitors, which display restricted lineage differentiation capacity, and finally produce lineage-restricted progenitors and mature cell types of the blood and immune system (see below).

Basic single-cell technologies, such as flow cytometry, colony forming assays, and single-cell transplantations, have been utilized for decades in the field of hematology [4]. These single-cell approaches have revealed extensive molecular and functional heterogeneity within the hematopoietic stem and progenitor cell (HSPC) compartment with regard to cell cycle activity, lineage bias, metabolic activity, or self-renewal capacity [5–8]. To account for the observed heterogeneity, a variety of subpopulations that enrich for particular cell states or phenotypes have been introduced [9–19]. Such a compartmentalization of the early hematopoietic system has proven highly valuable, but reinforced the notion that molecular and cellular changes along differentiation pathways and cell states typically occur stepwise. Single-cell analyses revealed that such populations still remain heterogeneous [19,

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20••, 21–27, 28••, 29]. The recent development of powerful single-cell omic approaches spanning the entire hematopoietic system has facilitated an ordering of the observed heterogeneity along differentiation trajectories, suggesting more gradual rather than discrete, step-wise transitions between cell states [30–34, 35••, 36••]. Together with functional single-cell and lineage tracing approaches, these studies have enabled a more comprehensive understanding of hematopoiesis, including new insights into lineage commitment hierarchies [5–8].

Large-scale single-cell approaches have also recently been exploited to study the bone marrow microenvironment responsible for maintaining HSCs and orchestrating their differentiation into mature blood cells [37–41]. These studies have provided fundamental insights into the niche composition and clarified some of the existing controversies in the field. Finally, powerful single-cell multi-omic technologies, where several entities of single cells such transcriptome, mutations, chromatin accessibility, and surface markers are measured in parallel, have recently been developed [42–48]. These technologies have significantly advanced our understanding of hematological malignancies deriving from HSPCs.

In the following, I discuss how recent single-cell (multi-)omic studies have transformed our understanding of normal and malignant hematopoiesis, as well as the bone marrow niche.

HSC Heterogeneity and Conflicts with the Classical Model of Hematopoiesis

Over the past years, single-cell approaches have revealed extensive molecular and functional heterogeneity within the HSPC compartment, conflicting with the traditional view of hematopoiesis [5–8]. In this section, I will briefly review the observed heterogeneity in the HSPC compartment, and introduce concepts of lineage-biased HSCs and transcriptional lineage priming. In the following section, I will set the observed heterogeneity in relation to newly introduced models of hematopoiesis.

Functional Heterogeneity of HSPCs

Functional heterogeneity of HSPCs has been recognized for decades [4]. In particular, differences in self-renewal capacities of HSCs have been studied in detail using transplantation of bulk subpopulations or single HSCs [2, 17–19, 20••, 21, 25, 27, 49–51]. Fluorescence-activated cell sorting (FACS) has been used to purify subpopulations that distinguish HSCs with high self-renewal capacity from MPPs. For example, the SLAM markers CD150 and CD48, the surface markers CD34 and Flt3, or EPCR have been used to identify HSCs with long-term self-renewal capacity, displaying the combined phenotype of Lineage⁻Sca1⁺cKit⁺CD150⁺CD48⁻Flt3⁻CD34⁻EPCR⁺ [13,

17–19, 52]. Combinations of these markers have also been used to define a plethora of MPPs with different self-renewal capacities, lineage biases, and molecular characteristics [10, 13]. In the classical model, self-renewal capacity is restricted to HSCs. However, elegant single-cell transplantation studies demonstrated that lineage-restricted progenitors present in the phenotypic HSC compartment (here: Lin⁻Sca1⁺cKit⁺CD34⁻) might exhibit high self-renewal capacities as well, exceeding those of some stem and multipotent progenitor cells [20••]. In particular, some transplanted phenotypic HSCs showed exclusive reconstitution of platelets, platelets and erythroid cells or platelet, erythroid, and myeloid cells with the absence of other lineages, both in primary and secondary transplantations. This observation challenged the strict mutual dependence of multipotency and self-renewal capacity. However, others have argued that this phenomenon might be restricted to the megakaryocytic lineage (see below) [53].

Moreover, single-cell transplantation and molecular barcoding experiments have demonstrated that the reconstitution patterns of individual HSCs are rarely identical [19, 21, 25, 51, 54–56]. In particular, certain lineages are frequently produced more abundantly than others. In this context, the term “lineage bias” has been introduced, referring to the relatively higher production of one or several lineages at the expense of the remaining lineages. The existence of megakaryocyte-, erythrocyte-, pan-myeloid- and pan-lymphoid-biased HSCs has been proposed [16, 18, 19, 20••, 29, 36••, 52, 57, 58]. It is important to note that a heterogenous lineage contribution of a single clone might be the consequence of an intrinsic lineage bias, but might also be imparted by the microenvironment, or simply reflect the consequence of the different kinetics of individual blood lineages [53]. Time-resolved lineage tracing approaches coupled to single-cell transcriptomic readouts will likely unravel the exact nature of HSC lineage biases [56].

Molecular Heterogeneity

In accordance with the existence of lineage-biased HSCs, the expression of transcriptional programs associated with lineage committed progenitors has been observed in individual cells of the phenotypic HSC compartment using single-cell transcriptomic profiling [17, 29, 30, 32–34, 35••, 59]. For example, von Willebrand factor and several other genes associated with the megakaryocytic lineage are expressed in a subset of HSCs [60]. This phenomenon has been termed “transcriptional lineage priming”. The use of indexed FACS surface markers common to both single-cell RNAseq data and single-cell ex vivo culture data has allowed to quantitatively link the direction of transcriptional lineage priming to functional properties [35••]. These analyses have revealed that the degree and direction of transcriptional lineage priming is quantitatively linked to the functional bias, with HSCs

displaying a certain type of transcriptional priming exhibiting a higher likelihood of commitment into the respective lineage [35••]. In line with heterogeneity in gene expression programs, a study measuring chromatin accessibility using single-cell assays for transposase-accessible chromatin by sequencing (ATACseq) revealed cellular variations among HSPCs at the regulatory level consistent with lineage biases towards different hematopoietic branches [61]. DNA methylation and chromatin modifications have so far been mainly studied at the bulk level in highly purified HSPC compartments [13, 62–65]. These analyses suggest that extensive heterogeneity might already be imprinted at the epigenetic level in HSC subsets. To address this point mechanistically, an elegant study made use of a mouse model in which individual HSC clones express unique combinations of fluorescent tags [66]. This permitted the simultaneous tracking of clonal fates at the functional level, as well as the mapping of transcriptional and epigenomic programs underlying the clonal heterogeneity. The study revealed that HSC function highly corresponds to its underlying epigenetic configuration under homeostatic and stress conditions [66]. In the future, combined readouts of transcriptome and epigenomic marks from single cells might be instrumental for a detailed rewiring of the cause-effect relationship between epigenetic remodeling and transcription in mediating HSC fate decisions [48, 67].

Cellular Heterogeneity

HSCs display pronounced heterogeneity in cellular processes, such as cell metabolism, autophagy, and cell cycle activity. The most potent HSCs are thought to depend on glycolysis, exhibit low mitochondrial respiration, and, consequently, produce low levels of reactive oxygen species (ROS) [68]. Importantly, a fraction of HSCs are maintained in a long-term quiescent state, termed dormancy, which is associated with a high self-renewal potential [14, 69]. Single-cell analyses in mouse and human have indicated that dormancy is associated with a biosynthetic inactive state and low translational activity [35••, 70]. Similar to the state of dormancy, high autophagy levels in HSCs have been associated with self-renewal capacity, a low metabolic state and quiescence [71, 72]. It is tempting to speculate that the states of dormancy and autophagy are directly linked.

In case of emergency, such as viral infections or blood loss, dormant HSCs are recruited into the cell cycle to accelerate blood production [14, 73–76]. HSC activation and differentiation is associated with increased metabolic activity, a switch to oxidative phosphorylation, elevated ROS production, and loss of self-renewal activity (Fig. 1).

Together, extensive molecular, cellular, and functional heterogeneity is observed in the HSPC compartment. While a large extent of this heterogeneity can be linked to the exit of dormancy, change of metabolic states, and preparation for

lineage commitment, additional sources of heterogeneity remain to be investigated.

Hematopoietic Landscapes Inferred from Single-Cell Omic Approaches

Large-scale single-cell transcriptomics spanning the entire hematopoietic system of the bone marrow have provided important insights into the organization of hematopoiesis, and enabled the translation of the observed heterogeneity into meaningful differentiation and cell-state trajectories (Fig. 1). While HSC and MPP populations have been conceptualized to form discrete cell types in the classical tree model of hematopoiesis, large-scale single-cell transcriptomic studies in mouse, zebrafish, and human have revealed that HSCs do not acquire transcriptionally stable, discrete cell stages upon lineage commitment, but rather gradually pass through a continuum of differentiation states [32–34, 35••, 54, 77]. In trajectory analyses of single-cell transcriptome data, HSCs initially upregulate transcriptional programs associated with lineage priming towards the megakaryocyte/erythrocyte/eosinophil/basophil versus the neutrophil/lymphoid direction, followed by branching into separate lineages and activation of lineage-specific transcriptional programs [32, 35••, 56, 78]. This pattern is observed not only in adult hematopoiesis, but also during embryonic hematopoiesis at the fetal liver stage [31]. Integration of single-cell culture or lineage tracing data with single-cell transcriptomic data suggested that early transcriptional lineage priming (megakaryocyte/erythrocyte/eosinophil/basophil versus the neutrophil/lymphoid priming) is associated with an increased likelihood of lineage commitment, whereas subsequent activation of lineage-specific transcriptional programs is linked to definitive lineage commitment [35••, 54, 56]. Importantly, lineage commitment occurs earlier than previously anticipated as revealed by single-cell molecular and functional analyses of progenitors downstream of HSCs [21, 22, 28••, 35••, 79]. These studies demonstrated that the majority of cells suggested to be oligopotent by the classical model of hematopoiesis (CMPs, MEPs, GMPs) have already acquired transcriptional lineage programs associated with functional lineage commitment [21, 22, 28, 35••, 79]. While oligopotent HSPCs and transcriptional mixed-lineage states do exist, they are much less frequent than suggested by the classical model [20–22, 35••, 80].

Highly similar findings that include the continuous nature of cell state transitions and branching hierarchies were made by measuring chromatin accessibility, instead of single-cell transcriptomes in large-scale single-cell ATACseq studies [48, 61]. To account for the continuous nature of hematopoiesis, continuum-based models have

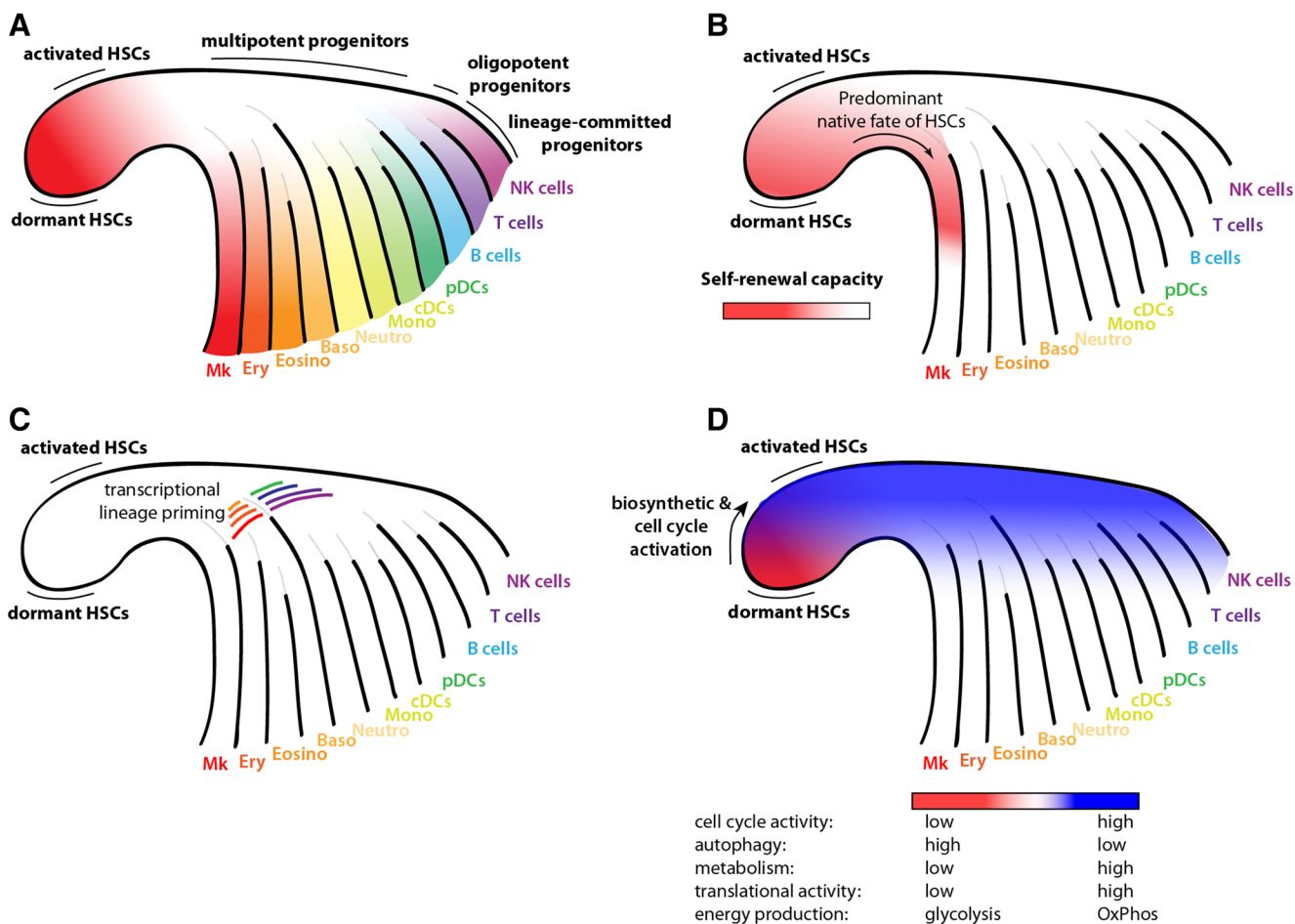


Fig. 1 Hematopoietic stem cell commitment model. **a** Overview. **b** Self-renewal capacity and indication of direct lineage commitment to the megakaryocytic lineage. **c** Indication of transcriptional lineage priming. **d** Indication of cellular activity. Mk megakaryocyte, Ery erythrocytes,

Eosino eosinophils, Baso basophils, Neutro neutrophils, Mono monocytes, cDC conventional dendritic cells, pDCs plasmacytoid dendritic cells, NK cells natural killer cells

been suggested that are capable of explaining single-cell data much more accurately [5, 7, 35, 61, 80]. In this framework, quiescent and metabolically inactive HSCs are maintained in a flat valley at the top of the hierarchy [35, 70]. Upon cell cycle induction, HSCs become biosynthetically active and subsequently acquire transcriptional lineage priming in a gradual manner. While in the early phases combinatorial lineage priming might be acquired, unidirectional priming becomes more prevalent over time, increasing the likelihood of future lineage commitment. Initially, barriers between lineages are small, allowing some plasticity. However, rapidly, transcriptional lineage programs associated with lineage commitment and cell-type manifestation are engaged.

Lineage Commitment Hierarchies

According to the classical model of hematopoiesis, the first bifurcation point into common lymphoid progenitor (CLPs)

and common myeloid progenitor (CMPs) separates lymphoid lineages (B, T, and NK cell lineages) from all myeloid, megakaryocyte, and erythrocyte lineages, followed by further sub-branching [2, 3, 11, 12]. This hierarchy was first revised when it was noted that megakaryocytes and erythrocytes branch off from lymphoid and myeloid progenitors, marked by a population termed lymphoid primed multipotent progenitor (LMPP) in mice [17] or multilymphoid progenitor (MLP) in human [81]. However, in these analyses, all myeloid lineages were investigated together and not individually. When myeloid lineages were investigated separately using molecular and functional single-cell approaches, it was noted that eosinophil and basophil lineages share common precursors with the megakaryocyte/erythrocytic lineages, whereas the neutrophil, dendritic cell, and monocyte lineages initially co-develop with lymphoid branches, followed by further sub-branching [79, 82, 83] (Fig. 1). This is in line with trajectory analyses from large-scale single-cell transcriptome data of HSPCs (see above).

Megakaryocyte Lineage Commitment—a Special Case?

A decade ago, it was already noted that despite their apparent phenotypic differences, HSCs and the megakaryocytic lineage share many common features [84]. These include commonly used transcription factors (e.g., Runx-1, Gata2, Evi-1, Tal-1), common surface receptors (e.g., c-mpl), and specific signaling pathways [84]. Later, it was shown that the phenotypic HSC compartment contains (i) megakaryocyte-biased HSCs that reside at the apex of the differentiation hierarchy [60•] and (ii) stem-like megakaryocyte progenitors that share many features with HSCs, exhibit high self-renewal capacity, but are committed to the megakaryocytic lineage [20••, 29]. An elegant study recently addressed the relationship between HSCs and the megakaryocytic lineage in unperturbed, native hematopoiesis [36]. For this purpose, the authors made use of transposon-based tagging of HSPCs combined with single-cell transcriptomic readouts to trace lineage relationships. This analysis revealed megakaryocyte commitment to be the predominant native fate outcome of HSCs (Fig. 1b). The close connection between HSCs and the megakaryocytic lineage might serve as an emergency pathway. For example, dramatic losses of platelets that might occur during infections induce rapid proliferation and differentiation of megakaryocyte-primed cells of the HSC compartment [29, 60•]. On the molecular level, stem-like megakaryocyte progenitors express Mk transcripts, but protein synthesis is suppressed during homeostasis. Upon inflammatory signaling initiation, translation of Mk transcripts is induced, mediating an emergency differentiation program that rapidly restores platelet levels [29].

Single-Cell Multi-omic Approaches in Hematological Disease

Leukemias frequently develop from healthy stem progenitor or precursor cells by a stepwise acquisition of genetic lesions, resulting in a differentiation block and accumulation of leukemia cells in the bone marrow. Understanding the complex clonal hierarchies in cancer is of great importance, since small sub-clones present at diagnosis might expand, and ultimately cause therapy resistance and relapse. While single-cell mutational profiling provides highly detailed maps of clonal hierarchies, combined single-cell transcriptomic and genomic assays offer the possibility of determining the molecular consequences of clonal evolution. In recent years, several methods that permit combined transcriptomic and genomic readouts have been introduced [42–47, 85], where mutations are called either indirectly from cDNA of scRNAseq data or separately from genomic DNA. Such approaches have been applied to distinct hematological malignancies, including acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPNs).

Chronic myeloid leukemia (CML) is an MPN typically driven by the oncogenic fusion of BCR and ABL1. In an elegant single-cell multi-omic study of CML, the Mead group combined high sensitivity BCR-ABL detection with whole transcriptome scRNAseq [43]. This allowed the discrimination of mutated CML cells at high precision and sensitivity, enabled the prediction of therapy response to tyrosine kinase inhibitors (TKIs), and led to the characterization of a quiescent CML stem cell population persisting throughout TKI therapy. In BCR-ABL-negative MPNs, the CALR gene has recently been described to be recurrently mutated [86]. To determine the effects of CALR mutations in MPNs, combined CALR genotyping and transcriptome analyses of thousands of single-cells has recently been performed using a novel high-throughput, droplet-based approach, termed genotyping of transcriptomes (GoT) [45]. This revealed that CALR mutations impart different effects on the transcriptome, dependent on the cellular state and disease type. For example, in patients with essential thrombocythemia, an MPN associated with an overproduction of platelets, CALR mutations resulted specifically in a pronounced cell cycle activation of mutant megakaryocyte progenitors [45].

To understand complex sub-clonal structures, protocols that allow the simultaneous mapping of many mutations in scRNAseq experiments have been developed and applied to bone marrow samples of MPN and AML patients [42, 44, 45, 47]. In AML, such studies have enabled the fine mapping of clonal and developmental differentiation hierarchies, revealed gene expression programs associated with pre-leukemic (e.g., TET2, SRSF2) and leukemic (e.g., CEBPA) mutations, and suggested that AMLs with monocyte-like phenotypes preferentially display immune modularity activity and T cell suppression [42, 44]. Importantly, computational pipelines for calling mutations directly from standard droplet-based scRNAseq approaches have been developed [85], facilitating multi-omic analyses without additional technical adaptations, albeit with much higher dropout rates if compared with specialized methods. Moreover, the possibility of tracking genetic clones by measuring mitochondrial mutations in single-cell genomic assays has been demonstrated, offering an elegant and efficient approach of tracing lineage relationships independent of genomic mutations [87].

Beyond mutations, a recent single-cell multi-omic study combined simultaneous measurements of transcriptomes, chromatin accessibility, and surface markers of single cells [48]. Using this approach, the authors generated maps of healthy hematopoiesis and mixed-phenotype acute leukemia. Integrative analyses identified putative transcription factors which regulate leukemia-specific expression programs.

Together, these single-cell multi-omic studies provide a framework for future integrative analyses and set the starting point for an in-depth understanding of hematological malignancies.

The Hematopoietic Stem Cell Niche at a Single-Cell Resolution

The bone marrow (BM) microenvironment, also referred to as the niche, plays a fundamental role in the maintenance and differentiation of HSCs [88, 89]. A multitude of niche cell types have been implicated in regulating HSC maintenance, including leptin receptor (*Lepr*) expressing cells, CXCL12 abundant reticular (CAR) cells, Nestin, and Ng2 expressing cells, as well as others [90–96]. The most intensively studied factors required for HSC maintenance in the BM are CXCL12 and SCF [90–92, 94–96]. Conventional approaches to study the niche typically make use of genetic labelling based on a single marker gene, in combination with genetic deletion of molecular factors, cell type ablation, or imaging. These approaches have provided fundamental insights into the contribution of niche cell types and cytokines required for HSC maintenance. However, the use of single genes for cell type definition has frequently resulted in the labelling of heterogeneous cell populations, and has therefore led to some controversies with fundamental questions remaining unaddressed.

Recently, single-cell transcriptomic studies from non-hematopoietic cells of the BM have elucidated the niche composition and clarified some of the controversies [37–41]. Notably, such studies permitted the unbiased identification and molecular characterization of known as well as previously unknown BM-resident cell types. For example, previously described CAR cells were shown to consist of subpopulations differing in their osteogenic versus adipogenic transcriptional priming, with the latter highly overlapping with previously described *Lepr* expressing cells. In line with previous studies, systematic assessments of cytokine synthesis across all BM cell types demonstrated that *Lepr*-expressing CAR cell populations were the main producers of CXCL12 and SCF, with relatively minor contributions of arterial endothelial cells. Remarkably, among all BM-resident cell types, CAR cell populations devote the largest proportion of their transcriptional activity to cytokine synthesis, suggesting that they act as professional cytokine producing cells [37].

Combining single-cell with laser-capture microdissection-based spatial transcriptomics allowed the systematic localization of BM cell types to distinct niches and enabled the spatial mapping of cytokine synthesis [37]. This approach demonstrated that CAR cell subsets differentially localize to either sinusoidal endothelia in the case of adipo-primed CAR cells, or to arterioles and non-vascular regions in the case of osteo-primed CAR cells. In line with this, *in situ* measurement of cytokine production demonstrated that the highest production of HSC factors occurs around both sinusoidal and arterial vessels. This suggests that unique niches are established by the differential localization of professional cytokine producing cells to specific regions in the BM. In line with previous

studies, these findings point to a particular importance of (peri)-vascular niches for the production of HSC maintenance factors. Interestingly, osteo-primed CAR cells were also found to localize to the trabecular part of the bone, suggesting a potential role in osteogenesis [40].

Together, single-cell studies of the BM niche have provided valuable insights into the HSC niche organization, and clarified the cellular and spatial sources of key HSC factors (see [41] for a detailed review). In the future, imaging mass cytometry and advanced spatial transcriptomic approaches, in combination with conventional approaches, could lead to a deeper understanding of the complex molecular and spatial organization of the bone marrow niche.

Conclusions

Single-cell approaches have considerably refined our perception of healthy hematopoiesis, hematological malignancies, and the bone marrow niche. In the future, integrative approaches should deliver multi-scale insights into the phenotypes, function, and spatial organization of the hematopoietic system at the single-cell resolution. Descriptive multi-scale phenotyping will include single-cell readouts at the transcriptome, genome, chromatin accessibility, DNA methylation, B cell and T cell receptor sequence, and surface proteome (CITE-seq, AB-seq-related technologies) level [48, 97–99], combined with lineage tracing approaches or pooled CRISPR screens in order to bridge descriptive phenotypes with mechanistic or functional data [56, 100–103].

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Compliance with Ethical Standards

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