Dynamic Changes of the Bone Marrow Niche: Mesenchymal Stromal Cells and Their Progeny During Aging and Leukemia

Kevin Woods¹,²,³ and Borhane Guezguez¹,²,³*

¹ German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany, ² Department of Hematology and Oncology, University Medical Center Mainz, Mainz, Germany, ³ German Cancer Research Center (DKFZ), Heidelberg, Germany

Mesenchymal stromal cells (MSCs) are a heterogenous cell population found in a wide range of tissues in the body, known for their nutrient-producing and immunomodulatory functions. In the bone marrow (BM), these MSCs are critical for the regulation of hematopoietic stem cells (HSC) that are responsible for daily blood production and functional immunity throughout an entire organism’s lifespan. Alongside other stromal cells, MSCs form a specialized microenvironment BM tissue called “niche” that tightly controls HSC self-renewal and differentiation. In addition, MSCs are crucial players in maintaining bone integrity and supply of hormonal nutrients due to their capacity to differentiate into osteoblasts and adipocytes which also contribute to cellular composition of the BM niche. However, MSCs are known to encompass a large heterogenous cell population that remains elusive and poorly defined. In this review, we focus on deciphering the BM-MSC biology through recent advances in single-cell identification of hierarchical subsets with distinct functionalities and transcriptional profiles. We also discuss the contribution of MSCs and their osteo-adipo progeny in modulating the complex direct cell-to-cell or indirect soluble factors-mediated interactions of the BM HSC niche during homeostasis, aging and myeloid malignancies. Lastly, we examine the therapeutic potential of MSCs for rejuvenation and anti-tumor remedy in clinical settings.

Keywords: mesenchymal stromal cells, bone marrow niche, aging, leukemia, adipocyte, osteoblast

Abbreviations: AML, Acute Myeloid Leukemia; B-ALL, B-cell Acute Lymphoblastic Leukemia; BM, Bone Marrow; CAR-cells, CXCL12-Abundant Reticular Cells; CML, Chronic Myeloid Leukemia; CMML, Chronic Myelomonocytic Leukemia; ECM, Extracellular Matrix; HSC, Hematopoietic Stem Cell; IHH, Indian Hedgehog; LEPR, Leptin Receptor; MDS, Myelodysplastic Syndrome; MF, Myelofibrosis; MIF, Macrophage Migration Inhibitory Factor; MNC, Mononuclear Cells; MPN, Myeloproliferative Neoplasm; MSC, Mesenchymal Stromal Cell; mTOR, mechanistic Target Of Rapamycin; NG2, Neural/glial antigen 2; PDGF-R, Platelet-Derived Growth Factor-Receptor; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PTH, Parathyroid Hormone; PuS, PDGF-Rα+/Sca-1⁺; SASP, Senescence-Associated Secretory Phenotype; SCF, Stem Cell Factor; TPO, Thrombopoietin; YAP, Yes-Associated Protein.
INTRODUCTION

Located within specific anatomical zones of the skeleton, the bone marrow (BM) is a specialized microenvironment or "niche" that lodges cells of hematopoietic and mesenchymal origins in various hierarchical committed states. The main role of the BM niche is the tight control of cell-fate decisions of the hematopoietic stem cells (HSCs) and their progeny to sustain the daily supply in functional blood and immune cells throughout life. These environmental cues are produced by a variety of stromal cells that constitute the BM niche which mainly include neurons, endothelial cells and mesenchymal stromal cells (MSCs) (Pinho and Frenette, 2019). The latter are considered a versatile stem cell population due to their capacity to differentiate into bone (osteoblasts), cartilage (chondrocytes) and fat cells (adipocytes), thus playing a central role in HSCs maintenance, BM niche composition and life-long turnover and bone growth (Bianco and Robey, 2015). Due to their fibroblastic nature and heterogenous origin, MSCs have been referred to in the literature under different names which were accounted for in this review. In addition, prominent gene reporter-mouse models that helped investigate the role of stromal populations in the BM led to synonymous use of the reporter strains themselves as putative markers for MSC populations, which are different from their human counterparts (see Table 1). However, current consensus divided MSCs into subgroups based on their anatomical location which influence both their functional and phenotypic potentialities. Therefore, within the scope of this review, we refer to the nomenclature proposed by Matsuzaki et al. (2014) and revised by Ambrosi et al. (2019); according to which MSCs are defined as bone marrow stromal cells bearing trilineage potential and expressing both Leptin receptor (LEPR) and PDGF-receptor α (PDGFR-α) in human and mouse (see Figure 1). Acknowledging the presence of further heterogeneity within the MSCs compartment, we will review major niche factors contributed by the MSCs and their osteo-adipo progeny in sustaining hematopoiesis. We will also present the most recent advances in identifying MSCs subset heterogeneity and cellular hierarchy by single cell technologies and their impact on remodeling the BM during aging and myeloid leukemias. Consequently, we will highlight possible therapeutic options in targeting MSCs in clinical settings.

FUNCTIONAL MSC HETEROGENEITY: LOCATION AND PROGENY MATTERS

The BM niche can be divided into two distinct regions based on the location of the cells, vascular flow and oxygen conditions they are exposed to which consequently define functional differences between MSCs within these distinct niche sites (see Figure 2):

The endosteal bone marrow niche represents 10% of total BM volume and comprises the MSCs with high osteolineage capacity including osteoprogenitors, osteoblasts, and osteocytes, which populate the inner surface of the bone along small arterioles and capillary vessels (Méndez-Ferrer et al., 2020). NG2⁺ pericytes and MSCs along with their osteo-progeny were shown to promote HSC quiescence through secretion of pro-survival and homing factors such as C-X-C Motif Chemokine Ligand 12 (CXCL12) (Wei and Frenette, 2018), Angiopoietin-1 (Ang-1) (Arai et al., 2004), thrombopoietin (TPO) (Yoshihara et al., 2007), and Notch ligands (Calvi et al., 2003; Guezguez et al., 2013) thereby reinforcing their tight contact with osteoblasts and maintaining the HSCs in a long-term non-cycling status (Qian et al., 2007; Loeffler and Schroeder, 2021). In accordance, the osteocalcin⁺ osteoblasts have been identified as a supportive “layer” niche due to their organization in follicle-like structures which surround HSCs and bind to them via N-cadherin- and Notch/Jagged1 mediated cell-cell interactions (Calvi et al., 2003; Zhang et al., 2003; Lawal et al., 2017). More recent reports indicate that the regulation of hematopoiesis by the osteolineage may also depend on its differentiation state (Sacchetti et al., 2007; Méndez-Ferrer et al., 2010; Calvi et al., 2012; He et al., 2017), as well as the close spatial localization of HSCs with the bone-lining cells of the endosteal niche (Lo Celso et al., 2009; Xie et al., 2009; Guezguez et al., 2013; Kim et al., 2017). These physical osteoblastic niche interactions controlling HSC fate are extensively influenced by a profusion of autocrine, paracrine, and endocrine factors such as bone morphogenetic proteins (Jung et al., 2008; Goldman et al., 2009; Khurana et al., 2014; Guo et al., 2018), growth factors (Yoon et al., 2012, 2017; Castelli et al., 2013), prostat glandins (Frisch et al., 2009; Hoggatt et al., 2009, 2013), shared cytokines/chemokines (Sugiyama et al., 2006; Ding and Morrison, 2013; Brylka and Schinke, 2019) and hormones such as the parathyroid hormone (PTH) (Calvi et al., 2001, 2003; Kuznetsov et al., 2004; Li et al., 2012). Although all of these molecules appear to be essential cornerstones for the preservation of bone microarchitecture and stem/progenitor cell homeostatic features within the BM, PTH has been identified as a key osteo-niche element linking MSCs and HSCs activities functionally and spatially (Adams et al., 2007; Li et al., 2012; Yu et al., 2012; Yao et al., 2014; Wein and Kronenberg, 2018). Additionally, osteoprogenitors were shown to be indispensable for B-cell differentiation by the release of Interleukin-7 (IL-7) and Insulin Growth Factor (IGF-1) which are critical for the maturation steps of B-cell progenitors (Wu et al., 2008; Yu et al., 2016). On the other hand, osteocytes were shown to restrict myelopoiesis by secreting granulocyte colony-stimulating factor (G-CSF) an important factor in HSC mobilization (Fulzele et al., 2013). The interdependence of endosteal BM niche inhabitants and the multifaceted signaling of MSCs and their osteo-lineage progeny in controlling HSC functions continue to be the object of intense investigation.

The central/perivascular bone marrow niche delineates 90% of total BM volume and englobes most of the vasculature that is enveloped with a variety of cells, including MSCs, pericytes, neurons along with adipocytes, which populate the central region of the bone shaft (Méndez-Ferrer et al., 2020). The BM vasculature in this region is enriched with arterioles that branch with thin-walled and fenestrated blood vessels called sinusoids. This endothelial architecture allows for the tight balance in the retention and activation of HSCs as well as the trafficking of their progenitors and mature immune cells back and forth the BM (Itkin et al., 2016). Along secretion of CXCL12, the LEPR⁺ MSCs enveloping the sinusoids are shown to produce stem cell...
Woods and Guezguez  Mesenchymal Plasticity in the Bone Marrow Niche

TABLE 1 | Nomenclature of stromal populations based on genetic/putative markers.

| Name | Used in | Refers to | Additional info | Organism |
|------|---------|-----------|----------------|----------|
| CAR-cell | Sugiyama et al., 2006 | Endosteal niche, near HSC, CXCL12-expressing cells | Not the same as PDGFR-α<sup>+</sup>/Sca1<sup>+</sup> cells, but both have trilineage potential (Helbling et al., 2019) | Mouse, Human (Aoki et al., 2021) |
| LEPR-MSC | Ding et al., 2012 | Scf-GFP expressing perivascular stromal cells | Express PDGF-R, CXCL12, not Nestin, perivascular niche | Mouse, Human |
| Mesenchymal stem cell | Jessop et al., 1994 | Stem cell with multilineage potential | | Mouse, Rat, Rabbit, Lamb, Human |
| Multipotent Mesenchymal stromal cell | Dominici et al., 2006 | CD105<sup>+</sup>, CD73<sup>+</sup>, CD90<sup>+</sup>, CD45<sup>−</sup>, CD34<sup>−</sup>, CD11b<sup>−</sup>, CD79a<sup>−</sup>, CD19<sup>−</sup>, HLA-DR<sup>−</sup> ISCT criteria | | Human |
| Nestin<sup>+</sup> MSC | Méndez-Ferrer et al., 2010 | Mesenchymal stem cells | Mouse model for MSC | Mouse, Human (Pinho et al., 2013) |
| NG2<sup>+</sup> pericyte | Kunisaki et al., 2013 | Pericytes that control HSC quiescence, different from LEPR<sup>+</sup> (sinusoidal) cells | Mouse model for MSC, also show trilineage potential | Mouse, Human (Kozanoglu et al., 2009) |
| PDGFR-α<sup>+</sup>-Sca1<sup>+</sup> MSC (PaS) | Morikawa et al., 2009 | Perivascular mesenchymal stromal cells | | Mouse |
| Skeletal stem cell | Abdallah et al., 2004; Bianco and Robey, 2015 | Mesenchymal stem cells | | Human |

FIGURE 1 | Nomenclature overview of different stromal populations including putative and gene markers and how they relate to MSCs. For the scope of this review, MSCs are defined as all colony forming cells that express both PDGFR-α and LEPR (Matsuzaki et al., 2014). PaS stand for PDGFR-α<sup>+</sup>/Sca1<sup>+</sup>. Figure was generated using Biorender.com.

factor (SCF, also known as KITL) that is required for long-term preservation of HSCs in the BM (Ding et al., 2012). Adipocytes, known to be a rich source in nutrients for the BM, also produce a variety of cytokines and factors involved in HSCs maintenance (SCF, IL-3, IL-6, CXCL12) (Kumar and Geiger, 2017) as well as inhibitors of hematopoiesis such as TGF-β1, a mediator of cell-cycle arrest (Scandura et al., 2004; Brenet et al., 2013) and lipocalin 2 (LCN2) that inhibits erythroid differentiation (Miharada et al., 2008). More intriguingly, accumulation of adipocytes as marrow adipose tissue (MAT) was also shown to reduce blood flow and suppress hematopoiesis through reduction of sinusoid caliber and microvasculature pruning (Scheller et al., 2016).

Overall, accumulating evidence has demonstrated a balance of MSCs differentiation commitment between osteoblastic and adipocytic lineages; as well as mutual dependency to ensure homeostasis that can be derailed during aging, chronic stress or cancer (Rendina-Ruedy and Rosen, 2017). However, possible
feedback signals between osteo-adipo lineage and their parental MSCs as well as their impact on BM niche biology remains to be elucidated.

SINGLE-CELL MSC HETEROGENEITY: LESSON FROM SINGLE CELL RNA SEQUENCING

MSC Heterogeneity in the Murine Bone Marrow
With the advance of single-cell RNA sequencing technologies (scRNA-seq), traditionally homogenous cell populations reveal functionally different subclasses. The same is true for MSCs; recent well-designed scRNA-seq studies from different stromal gene-reporter mice shed some light on the murine bone marrow and help us to identify subclasses of MSCs. However, results from these studies varied greatly in number of identified MSC subsets due to different methods of BM extraction, cell sorting and sequencing depth (see Table 2). In summary, both “adipogenic” and “osteogenic” clusters can be identified regardless of the gene-reporter or surface MSC marker (LEPR+, CD51/-/Sca1+, PDGFRα+, Col2+) (Tikhonova et al., 2019; Wolock et al., 2019; Baccin et al., 2020; Zhong et al., 2020). Depending on gene set signatures, MSC can be subdivided into subsets with less differentiated and more stem-like features that are defined as mesenchymal progenitors or mesenchymal stem cells (Tikhonova et al., 2019; Zhong et al., 2020). Additionally, some of these studies also discerned “intermediate” MSC populations, suggesting that adipogenic and osteogenic differentiation is a continuous process with little definite cell states in-between (Tikhonova et al., 2019; Wolock et al., 2019; Leimkühler et al., 2021), as shown recently for the HSC compartment (Liggett and Sankaran, 2020).

MSC Heterogeneity in the Human Bone Marrow
There are few comparable scRNA-seq studies of the MSC heterogeneity in human. This is in parts due to the scarcity of material and the difficulties in getting consistent cell content from BM aspirates. Compared to full mouse bones, human BM aspirates contain very few MSCs within the range of 0.001–0.01% of total cellularity (Pittenger et al., 1999; Qin et al., 2021). In addition, the donors’ age and sex also influences MSCs phenotype and content (Siegel et al., 2013), adding another layer of heterogeneity to the analyzed samples. Further approaches to increase MSCs content from human material require enrichment applications by cell sorting strategies and in-vitro expansion, inevitably leading to a loss of subpopulations and altered gene expression while affecting resolution capacity of scRNA-seq (Ghazanfari et al., 2017; Liu et al., 2019). The current high cost of single cell-sequencing and the low MSCs content typically result in scRNA-seq experiments with fewer than 100 MSCs, resulting in difficulty for sub-clustering analysis.
In consequence, these experiments translate BM-derived MSCs as a single “homogenous” population that is compared to other MSC sources (Barrett et al., 2019; Zhou et al., 2019). In a recent scRNA-seq mapping experiment of large BM hematopoietic cell populations, a small amount of heterogeneous MSCs were captured, with one subset expressing high levels of the key bone marrow-homing cytokine CXCL12. This MSC subclass was later validated by high enrichment of CXCL12 and other key MSC

| Tissue obtained                                      | Sorted on and digested | Single method                  | Stromal population | Subclasses               | Signature genes          | Number of cells | References                        |
|------------------------------------------------------|------------------------|--------------------------------|-------------------|--------------------------|--------------------------|-----------------|-----------------------------------|
| BM flushed, bones crushed and digested withSTEMzyme1, Dispase II, ACK lysis | CD71<sup>-</sup>/CD45<sup>-</sup>/CD3<sup>-</sup>/B220<sup>-</sup>/CD19<sup>-</sup>/Gr-1<sup>-</sup>/CD11b<sup>-</sup> | Chromium single cell 3' Reagent V2 (10x genomics), Chromium Controller (10x Genomics) | LEPR<sup>+</sup> | N/A | LEPR<sup>E</sup>, CXCL12<sup>mt</sup>, KitL<sup>Y</sup>, Grem1<sup>hi</sup>, Angpt1<sup>mt</sup> | 20.896 | Baryawno et al., 2019 |
| BM flushed, bones digested with Lepr-tsT<sup>+</sup> and DNAaseI | Lepr-tsT<sup>+</sup> | Chromium single cell 3' Reagent V2 (10x genomics), Chromium Controller (10x Genomics) | LEPR<sup>+</sup> | N/A | LEPR<sup>E</sup>, CXCL12<sup>mt</sup>, KitL<sup>Y</sup>, Grem1<sup>hi</sup>, Angpt1<sup>mt</sup> | N/A | N/A |
| BM flushed, bones digested with Liberase<sup>TM</sup> and DNAaseI | CD45<sup>-</sup>/Ter119<sup>-</sup>/CD31<sup>-</sup> | inDrops (Weitz et al., 2015) | CD51<sup>-</sup>/ Sca1<sup>+</sup> | Pre-Adipocyte/Adipocyte progenitor | Nr4a1, CXCL1, Ifrd1, Fosb, Co2, LEPR, Kitl, Adipoq | 17.374 | Tikunova et al., 2019 |

| MSC                                                                   | Cb1n1, Clec2d, Pdzrm4, Cypb, Rspo2, LEPR, CXCL12, Kitl, Adipoq |
| Osteoblast/chondrocyte progenitor                                      | Postn, Wif1, Mmp9, Kcnk2, Limch1, LEPR, CXCL12, Kitl, Adipoq, Alpl, Col1a1, MMP13, Spp1 |
| Pre-osteoblast/chondrocyte                                            | Postn, Wif1, Mmp9, Kcnk2, Limch1, Alpl, Sp7, Col1a1, Mmp13, Spp1 |
| Pro-osteoblast/ chondrocyte                                           | Col1a1, Bgp1, Col11a2, Col11a1, Bgp2p2, Alpl, Sp7, Col1a1 |
| Pro-chondrocyte                                                       | Dmp1, Ackr3, Spp1, Ank, CD44, Col1a1, Mmp13, Mepe, Spp1 |

(Continued)
TABLE 2 | Continued

| Tissue obtained                        | Sorted on MACS separation (CD5−, CD45R−, CD11b−, Ly-6G/C−, 7−, Ter-119−) | Single cell method | Stromal population | Subclasses | Signature genes | Number of cells | References           |
|----------------------------------------|---------------------------------------------------------------------------|--------------------|--------------------|------------|-----------------|-----------------|---------------------|
| Bones crushed, cells filtered,         | CD41−, CD3−, CD11b−, Gr1−, Ter119−, CD45R−, CD45.1−, CD45.2−, Sca1−, CD31−, CD33−, CD51− | Chromium single cell 3′ Reagent V2 (10x genomics) | LEPR+, PDGF-R-α+, Vcam1+, CXCL12+, Kit1+, Angpt1+ | Adipogenic | Mgp, Adipoq, CXCL12, Kit1 | 2.294 | Leimkühler et al., 2021 |
| bone chips digested with Collagenase Il/Dispase, filtered, ACK lysis, lineage depletion (Dynabeads) | Ter119−, CD41−, CD45−, CD51−, CD71−, VCAM1−, CD200−, CD61− | Chromium single cell 3′ Reagent V2 (10x genomics) | PDGF-R-α+ | Adipo-CAR | Cxcl12, Tmem176b, Hp, Lpl, Tmem176a, H2-D1, Apoe, Gas6, Adipoq, Esm1 | 7.497 | Baccin et al., 2020 |
| Bones scraped to remove periosteum, bones flushed, bone chips digested with proteases | Co2-Td+ | Chromium Controller V3 (10x genomics) | Co2 | Early mesenchymal progenitors | Ly6a, CD34, Thy1, Mfap5, Gsn, Clec3b | 7.585 | Zhong et al., 2020 |
|                                        |                                     |                    |                    | Late mesenchymal progenitors | Apn, Edl3, Tnn, Postn, Ostn, Dkk3 |
|                                        |                                     |                    |                    | Osteoblasts/Osteocytes | Sp7, Runx2, Col1a1, Lbsp, Bglap2, DMP1 |
|                                        |                                     |                    |                    | Adipocytes | Cebpa, Cebpβ, Pparγ, Lpl, Adipoq, Apoe |
|                                        |                                     |                    |                    | Chondrocytes | Sox9, Col2a1, Col10a1, Pth1r, Acan, Ihh |
signature genes from FACS-based isolation of CD13+CD11a− cells (Triana et al., 2021). Another notable exception is a study done by Wang et al. (2020), where a total of 14.494 CD271+ BM-MNCs were analyzed. This study led to similar findings compared to the murine experiments, revealing adipo-, osteo-, and chondrogenic clusters as well as two terminal clusters that could represent senescent cells (see Table 3).

Recent advances in species transfer learning methods allowed the harmonization of single cell-sequencing data from mouse to human, finding equivalent clusters of cells in BM of both species (Stumpf et al., 2020). While this approach is useful to generalize findings across species, it is also limited in several ways, e.g., only orthologous genes are transferred. Even within the same cluster of cells of each respective species, there are significant transcriptional profile differences, for instance in GO terms (Wang et al., 2020), posing the question whether these cells truly play the same role in mouse and man. With all these factors in mind, we propose the following hierarchy of the MSCs and their progeny in the BM that is validated in both mouse and human (see Figure 3), with the outlook that future studies will reconcile the missing phylogenetic gaps for a unified cellular portrait of MSCs.

**MSC CHANGES IN AGING BONE MARROW**

During aging, the BM undergoes drastic changes with loss in osteoblasts and increase in adipocytes content leading to a change in overall cellularity, bone density and a shift in anatomical distribution from “red” to “yellow” marrow (reviewed in detail by Goltzman, 2019). In recent years, focus has been set on MSCs as the main source of these changes with the hope of ameliorating age-related alterations such as osteoporosis. In accordance with age-shift toward an adipogenic phenotype, recent scRNA-seq studies in old mice found that MSC subsets with adipogenic potential (AdipoCAR) increase excessively alongside with a depletion of mature osteoblasts (Zhong et al., 2020; Dolgalev and Tikhonova, 2021). However, there are conflicting reports about the overall number of MSCs during BM aging, with some studies indicating no changes (Aguilar-Navarro et al., 2020; Meza-León et al., 2021) while a majority of reports indicates an increase in some subsets of MSCs (Maryanovich et al., 2018; Frisch et al., 2019; Singh et al., 2019). These discrepancies can be explained due to different methodological approaches and is further underlined by pathological observations demonstrating divergent cellular BM changes between mouse and human during aging (Meza-León et al., 2021). However, common mammalian features of functional deregulation have been described in deciphering the age-related changes of MSCs:

**Direct Deregulation**

The observed hypocellularity in aged individuals can be attributed to altered MSCs differentiation capacity toward expansion of adipocytes and increased risk of osteoporosis. Indeed, MSC show an age-dependent lineage switch between the osteogenic and adipogenic fate. Under normal conditions, MSCs homeostasis is regulated by transcription factors PPARγ and C/EBPs toward the adipogenic lineage and Runx2 and Osterix for the osteogenic lineage. These in turn are controlled by cell adhesion toward extracellular matrix (ECM)-Integrins and molecular signaling from Wnt, Notch, BMP, Hedgehog and FGF pathways (Figure 2 and reviewed in detail by Chen et al., 2016). In consequence, these pathways are of special interest to identify aging effects. Clinical data demonstrated that patients with osteoporosis or age-dependent bone loss display low activity of Wnt/β-Catenin signaling in MSC while RhoA-Rock activity is inversely correlated with β-Catenin signaling in BM-MSCs from elderly human subjects (Stevens et al., 2010; Shi et al., 2021). The decrease of Wnt-signaling can be attributed in parts to a decrease in Yes-associated protein (YAP) in MSCs during aging, a co-transcription factor that was identified recently as an interaction partner of the β-Catenin complex (Pan et al., 2018). Recent studies revealed additional transcriptional regulatory mechanisms of the Wnt pathway by different classes of non-coding RNAs, such as microRNA miR-146a, whose levels increased in patients suffering from bone fragility (Saferding et al., 2020). Other circular (Ji et al., 2021) and long (Li et al., 2018) non-coding RNA were also found to play a role in lineage commitment by inhibiting the Runx2 transcriptional complex needed for osteoblastic differentiation. The delicate balance between osteo- and adipogenesis via the different transcriptional programs can also be influenced by Bmi1, a polycomb group protein that restricts adipogenic differentiation (Kato et al., 2019) and is downregulated in aged mice (Zheng et al., 2021). Similar to Wnt pathway, Indian Hedgehog-(IHh) signaling, which induces chondrogenesis in human MSCs (Steinert et al., 2012), was shown to be decreased in peroxide-induced senescent MSCs and MSCs from older donors (Al-Azab et al., 2020). Furthermore, adipogenesis and osteoclastogenesis is promoted indirectly by Sirtuin 3 (Sirt-3), a metabolic regulator of cellular senescence driven by the mTOR-pathway, that is found to be elevated in aged male mice and resulting in cortical bone loss (Ho et al., 2017).

**Senescence**

Besides an apparent increase in MSCs content during aging, there is also a substantial increase in their senescence contributing to a decrease in the osteoblastic lineage and accelerated bone loss. A possible reason for this might be the development of age-dependent inflammatory niche signaling, leading to noticeably increased IL-1α levels (which induces senescence via Bmi-1 downregulation) as well as IL-6 and TGF-β (Valletta et al., 2020; Zheng et al., 2021). A wide range of non-coding RNA have also been shown to regulate senescence both in mice and human (reviewed in Cai et al., 2021). In addition, aged MSCs produce high amounts of CXCL2 and CXCL5 chemokines, which contribute to the senescence-associated secretory phenotype (SASP) (Helbling et al., 2019). RANKL, an osteoclastogenic cytokine, has been shown to be increasingly secreted by MSCs in aged mice (Lin et al., 2017), leading to bone loss (Kim et al., 2020). Cellular senescence also leads to a decrease in Optineurin (OPTN), an autophagy receptor therefore contributing to osteoporosis alongside with accumulation of the OPTN substrate fatty acid binding protein 3 (FABP3) (Liu et al., 2020).
TABLE 3 | Human MSCs subsets based on sc-RNA sequencing of human BM tissue.

| Tissue obtained | Sorted on | Single cell method | Stromal population | Subclasses | Signature genes | References |
|-----------------|-----------|--------------------|--------------------|------------|----------------|-----------|
| Bone marrow aspirate, density gradient (Ficoll 1.077), lysis, CD271+ MACS separation (Miltenyi) | No sorting | Chromium single cell 3′ Reagent V2 (10x genomics) | LEPR+ | Osteogenic | XIST, COL6A3, COL1A1, VCAN, C7, THY1, ADM, ANGPTL4, PGF, COL6A2 | Wang et al., 2020 |
|                 |           |                    |                    | Adipogenic | HP, IGHG3, IGKC, FBLN1, RETRE31, APOD, CTGF, ADIPOQ, MGP, RPS26 |           |
|                 |           |                    |                    | Terminal 1 | FTL, RPS12, RPL30, RPS3A, RPL10, RPL34, TPT1, RPL12, RPS4X, RPS24 |           |
|                 |           |                    |                    | Terminal 2 | XIST, MALAT1, CSAD, NKTR, KCNQ1OT1, FUS, GOLGB1, WS81, CCNL2, CCNL1 |           |
|                 |           |                    |                    | Chondrogenic | S100A8, S100A9, S100A12, CAMP, LTF, MND, S100A4, MMP9, LCN2, LYZ |           |

Indirect Deregulation
A possible mechanism for the observed increase in MSCs might be driven by sensory adrenergic denervation that occurs in the aging microenvironment (Neuropathy), which in turn leads to reduced negative regulation of MSCs pool size and to the expansion of certain subsets holding adipogenic potential (Maryanovich et al., 2018; Ho et al., 2019). These shifts in BM content are further exacerbated by an increase in endothelial cell numbers and a regression of arteriolar structures (Kusumbe et al., 2014). Such BM stromal transformations increases the risk toward a myeloid-skewing differentiation of HSCs and can potentially lead to clonal hematopoiesis and subsequent hematological neoplasia (Steensma and Ebert, 2020).

The aforementioned changes in the MSC niche are summarized in Figure 4.

Addressing Age-Related Changes in the Niche
In recent years, focus on reverting cellular senescence became of major interest in addressing the aging-associated changes of MSCs. These approaches involve targeting the metabolic regulators Sirtuins 1 and 3 (Ma et al., 2017, 2020), pro-longevity growth factors such as fibroblast growth factor 21 (FGF-21) (Li et al., 2019) and downstream targets of HIF1α such as macrophage migration inhibitory factor (MIF) (Xia et al., 2015). A recent promising target is the hormone Lipocalin-2 (LCN2) that was previously shown to have a beneficial role in the regulation of various aspects of energy metabolism, especially in promoting fatty acid oxidation (Guo et al., 2010; Paton et al., 2013; Zhang et al., 2014). Further studies demonstrated that overexpression of LCN2 protect MSCs against stress-induced senescence and improve their paracrine and regenerative potentialities (Halabian et al., 2013; Bahmani et al., 2014). Furthermore, an LCN2 transgenic mouse model driven by bone-specific type 1 collagen, an osteolineage-specific promoter, showed expansion of long-term HSCs with higher clonogenic capacity due to elevated levels of CXCL12, SCF and matrix metalloproteinase inhibitors released by the BM niche (Costa et al., 2017). It has also been shown that osteoblasts, which decrease during aging, are the major source for blood circulating LCN2 in the body (Mosialou et al., 2020). Taken together, these findings suggest a beneficial effect of LCN2 supplementation on promoting hematopoiesis and stabilizing the aging BM microenvironment that would require further investigation for potential therapeutic applications.

In parallel, rewiring the MSC differentiation balance, originally explored as a rejuvenation strategy for treating
osteoporosis, is currently under investigation as potential regenerative therapy to restore healthy hematopoiesis. One major example is the intermittent treatment with PTH or PTH-related peptide (PTHrP), shown to exert a well-known anabolic effect on the skeleton (Osagie-Clouard et al., 2017) and induction of HSC expansion (Calvi et al., 2003; Adams et al., 2007). Further studies demonstrated that Nestin⁺ MSCs isolated from PTH-treated mice displays enhanced proliferation and differentiation into osteoblasts in culture (Méndez-Ferrer et al., 2010; Ding et al., 2012); as well as increased osteogenic differentiation capacity in vivo (Fan et al., 2017). Other studies based on drug screening of natural senolytic substances such as Celastrol and Quercetin 3-O-β-D-galactopyranoside was also shown to promote osteogenesis and inhibit adipogenesis in vitro through PGC-1α signaling (Li et al., 2020; Oh et al., 2020). On a similar note, inhibition of the mTOR-pathway was shown to extensively prolong life-span in mice (Papadopoli et al., 2019), including revitalized pluripotency of human MSCs in vitro (Antonioli et al., 2019). Epigenetic modifiers were also recently proposed to revert the fat-bone-imbalance in skeletal aging, especially Lysine Demethylase 4B, which was shown to regulate β-catenin/Smad1 signaling toward MSC rejuvenation (Deng et al., 2021). Lastly, rejuvenated MSCs could also be interesting for ex vivo HSCs expansion in the context of stem cell transplantation therapies. As such, a recent and elegant co-culture study of HSCs with MSCs allowed to identify a set of “rejuvenating” transcription factors (Klf7, Ostf1, Xbp1, Irf3, and Irf7), that when over-expressed in MSC induces expansion of HSCs with enhanced regenerative
and engraftment capacity while preventing accumulation of DNA damage (Nakahara et al., 2019).

In summary, most of these anti-aging approaches will require further validation prior possible translation toward clinical applications and other stromal targets not cited in this review are also currently under investigation (reviewed in more detail by Meng et al., 2020).

**MSC HETEROGENEITY IN MYELOID MALIGNANCIES**

Myeloid malignancies are clonal blood diseases arising from HSCs or subsequent progenitor cells that acquired oncogenic mutations and/or chromosomal translocations over a period of several years. Depending on the etiology of the disease, myeloid malignancies comprise chronic stages (including myelodysplastic syndromes: MDS, myeloproliferative neoplasms: MPN and chronic myelomonocytic leukemia: CML) and acute stages encompassing different subtypes of Acute Myeloid Leukemia (AML) (Arber et al., 2016; Sperling et al., 2017; Vetrie et al., 2020; Witkowski et al., 2020). A large body of work demonstrated direct and indirect involvement of the BM niche in supporting neoplastic and leukemic cells during the development of myeloid malignancies. These tumorigenic features include advantageous release of pro-survival factors, competition in niche space with healthy HSCs, stromal reprogramming and physical protection against therapy (Méndez-Ferrer et al., 2020; Witkowski et al., 2020).

**Msc Niche-Driven Hematological Malignancies**

Genetic mutation in mouse models affecting MSCs or their osteolineage progeny can induce different types of myeloid malignancies. For instance, activating-mutations in Nestin+ MSCs of the protein tyrosine phosphatase SHP2 (a positive regulator of the RAS signaling pathway) can lead to the development of childhood-like MPN by hyperactivating HSCs via overproduction of the CC-chemokine CCL3 and IL-1β (Dong et al., 2016). By contrast, deletion of the microRNA regulator DICER-1 in the Osterix+ osteolineage cells, prompt a pre-leukemia disease that mirrors human MDS and can evolve into secondary AML (Raaijmakers et al., 2010). Similarly, induction of Shwachman-Diamond syndrome mutation in Osterix+ stromal cells was shown to drive MDS evolution through the S100A8/9-TLR inflammatory signaling axis as a common driving mechanism of genotoxic stress that predicts AML progression in human patients (Zambetti et al., 2016). More recently, osteoblasts have also emerged as critical drivers of MDS via activating mutations in β-catenin signaling that can lead to progression to overt AML in mice (Kode et al., 2014; Stoddart et al., 2017). This aberrant activation of β-catenin signaling is also found in stromal cells of MDS patients along with DICER-1 dysregulation (Santamaría et al., 2012; Ozdogan et al., 2017) correlating with adverse prognosis (Bhagat et al., 2017).

**MSC Niche Reprogramming by Leukemia**

Neoplastic and malignant cells can further remodel the MSC niche by specifically targeting the osteoblastic progeny during the stepwise disease progression from pre-leukemia stage (MDS/MPN) to overt AML (Yamaguchi et al., 2021). Specifically, it was shown that both MDS and MPN cells secrete inflammatory mediators such as CCL3 and TPO, thereby driving transformation of the MSC niche toward a highly supportive milieu for leukemic cell expansion at the expense of normal hematopoiesis (Scheper et al., 2013; Medyouf et al., 2014). This is consistent with xenograft studies suggesting that the MSC niche also provides a chemo-resistant niche for leukemic blasts (Ishikawa et al., 2007; Duan et al., 2014; Bertoli et al., 2018; Boutin et al., 2020).

Healthy Nestin+ MSCs and osteoblasts can also be indirect targets of sympathetic neuropathy (through β2-adrenergic
signaling) in models of myeloid malignancies, leading either to aberrant expansion or loss of Nestin+ MSCs while restricting the numbers of mature osteoblasts in both MLL-AF9-AML (Hanoun et al., 2014) and JAK2V617F-MPN mouse models (Arranz et al., 2014). As a result, the impaired MSC niche promotes expansion of mutant HSCs and facilitates disease progression by loss of expression of HSC-retention factors, including CXCL12, SCF, ANG1, and VCAM1 (Arranz et al., 2014; Hanoun et al., 2014). Collectively, this is in agreement with clinical observations of stromal cells from MDS/AML patients, where expression of cell-surface molecules involved in interaction with HSCs is decreased (Geyh et al., 2013), whereas the population of human MSCs is increased, favoring blast expansion (Kim et al., 2015). In addition, osteogenic differentiation is significantly impaired by remodeling of the vasculature leading to reduced osteocalcin serum levels and deficiency in bone growth (Geyh et al., 2016; Duarte et al., 2018; Kumar et al., 2018), which is in line with reports of osteopenia or osteoporosis observed in newly diagnosed children or adults with acute Leukemia (Datzmann et al., 2018; Ruchlemer et al., 2018; Ahn and Suh, 2020).

Mapping MSC Niche Heterogeneity in Leukemia

Despite the multiple functional studies investigating the role of the BM niche, little is known on the extent of transcriptional reshaping of the MSC populations in myeloid malignancies, but recent scRNA-seq studies led to a better understanding of lineage shift and disease specificity. In AML context, single cell data revealed a concomitant decrease in committed osteolineage LepR+MSCs in an MLL-AF9 mouse model along with an increase in pre-osteoblasts, suggesting a block in osteolineage maturation (Baryawno et al., 2019). This osteogenic differentiation blockade was further accompanied by a loss of transcriptional expression of multiple HSC niche factors (Vcam-1, CXCL-12, SCF, Angpt, Il-7, CSF1) and gene expression changes were also observed in endothelial cells and adipocytic populations (Baryawno et al., 2019). In a similar manner, RNA-seq studies on BM stroma from both mouse and human MPN shed light on the functional contributions of individual cellular components of the MSC population to myelofibrosis (Leimkühler et al., 2021). ScRNA-seq analysis demonstrated a fate switch between distinct precursor cells and MSC populations during stress-injury induced by malignant MPN clones. Two distinct MSC populations were shown to be the main drivers of BM fibrosis in mouse and human MNP. These two MSC populations are of LepRβ+ origin and are either adipogenic or osteogenic-biased progenitor populations. During MPN disease evolution, these MSC populations were demonstrated to be functionally reprogrammed into Collagen-producing myofibroblasts, reminiscent of Gli-1+ fibrosis-driving cells (Schneider et al., 2017) and leading to the excess deposition of ECM in BM which is considered one of the hallmarks of overt myelofibrosis (Barbui et al., 2018). Interestingly, all other MSC subsets were also shown to be reprogrammed into the production of non-collagenous ECM with scaffolding function for collagen fibrosis. This aberrant lineage shift was due to increased stromal expression of chronic inflammatory signals, especially TGF-β and S100A8/S100A9, leading toward a loss of hematopoiesis support (Vogl et al., 2018; Ribezzo et al., 2019).

Although more effort is necessary to unravel the MSC changes in different myeloid malignancies stages, all functional and genetic data indicate a shift toward an accumulation of MSC with adipogenic potential (Figure 4) that might be instrumental in disease evolution and should be explored further to specify therapeutic targeting.

Development of MSC Therapies for Myeloid Malignancies

Given the central role of MSCs in the maintenance of both HSC and leukemic blasts, numerous studies investigated their potential direct therapeutic use in hematopoietic malignancies such as MDS and AML (reviewed in Fathi et al., 2019; Lee et al., 2019). Early co-culture studies of MSC and leukemia cells displayed contradictory results: either increased blast survival (Garrido et al., 2001) or anti-leukemic effects through the induction of apoptosis and cell cycle arrest (Liang et al., 2008; Tian et al., 2010). More broadly, a direct use of MSCs as a cellular anti-cancer therapy also proved to be difficult since the cells do not survive long enough to exhibit any beneficial effects (Levy et al., 2020) and were even shown to promote tumor growth in mouse models of MLL-AF9 AML and metastatic solid cancers (Okumura et al., 2009; Spaeth et al., 2009; Xu et al., 2009; Hanoun et al., 2014).

Acknowledging this functional duality of MSCs in leukemia growth, further research was directed in developing antibodies or compounds that target specifically the supportive malignant cues, more prominently toward the inhibition of the CXCL12-CXCR4 axis (Zhang et al., 2012; Kuhne et al., 2013) and IL6 signaling (Stevens et al., 2017). These promising compounds are currently being tested in combination with standard chemotherapy or allogeneic transplantation settings in clinical trials of high-risk MDS and refractory AML patients (Martínez-Cuadrón et al., 2018; Roboz et al., 2018; Michelis et al., 2019; Bose et al., 2020). On the other hand, the anti-tumoral effects displayed by MSCs were attributed to small secreted factors (Maguire, 2013; Moll et al., 2020; Wu et al., 2020) and led to increased interest in the use of MSC secretome for anti-leukemic therapy as well as for a wide array of other diseases, such as ischemic, neuroinflammatory and pulmonary malignancies (reviewed in Harman et al., 2021). Collective proteomic studies demonstrated that the MSC secretome consists of trophic factors (e.g., FGF, HGF, VEGF), cytokines (e.g., IL-6, TGFβ1–3), hormones, small peptides (e.g., SCF, PTG, Leptin) and extracellular vesicles (EVs) containing miRNA, mRNA and biologically active proteins (Chulpanova et al., 2018). In consequence, cell-free therapy options are considered more promising for clinical applications (Hmadcha et al., 2020). However, it was shown that EVs from MSC can also contribute to tumor cell migration and growth by activation of Wnt, Erk or Akt pathways (Lin et al., 2013; Gu et al., 2016; Shi et al., 2016). EV content is dependent on many factors, such as MSC source (adipose tissue, umbilical cord, bone marrow), donor age, individual donor-specific influences,
increased over the last decades, more knowledge is required.

MSC niche contributions to aging and Leukemia has hugely inducing chemoresistance. Although our understanding of malignancies by protecting cancer cells from apoptosis and as demonstrated by biochemical interactions leading to the development of a proinflammatory environment.

modifications of both the morphology and functions of MSCs, cytokines and EVs. The aging process imposes profound secretion of a wide variety of factors, such as growth factors, or infection. These activities are carried out through the modulation of the immune system response during injury homeostasis, the support of the hematopoietic niche and the exerting multiple functions that are fundamental for tissue MSCs represent a key component of the BM microenvironment, while eliminating tumor-promoting effects.

CONCLUDING REMARKS

MSCs play a key role in the BM microenvironment, exerting multiple functions that are fundamental for tissue homeostasis, the support of the hematopoietic niche and the modulation of the immune system response during injury or infection. These activities are carried out through the secretion of a wide variety of factors, such as growth factors, cytokines and EVs. The aging process imposes profound modifications of both the morphology and functions of MSCs, leading to the development of a proinflammatory environment. Increasing evidence demonstrate that this reshaping of the MSC niche is exacerbated during disease progression in hematologic malignancies by protecting cancer cells from apoptosis and inducing chemoresistance. Although our understanding of MSC niche contributions to aging and Leukemia has hugely increased over the last decades, more knowledge is required to harness the depth of complex MSC interactions with the highly polyclonal nature of aberrant HSCs or leukemic cells driving disease heterogeneity in MDS/AML. Moreover, many questions remain unresolved; in particular, whether the phenotypes and molecular mechanisms identified in vivo or in mouse models are maintained and therapeutically relevant in the human disease. In addition, the use of human leukemia samples in understanding aberrant MSC niche biology is currently hindered as clinical standard diagnoses are made on BM aspirates that disrupt BM architecture. Recent developments in single-cell sequencing and imaging technologies have made it possible to assess the heterogenous composition and diverse cellular and biochemical interactions present throughout complex tissue. Future integrative single-cell studies aimed at identifying the diverse network of cellular and biochemical interactions underlying the MSC niche may uncover unappreciated regulators or pathways controlling the BM aging process and cancer reprogramming and could lead to the development of novel therapeutic strategies aimed at improving health of the aging population or tackle chemoresistance in hematological malignancies.

AUTHOR CONTRIBUTIONS

BG and KW designed and edited the figures and tables. Both authors contributed to the manuscript.

FUNDING

This work was funded by the support of German Cancer Consortium (DKTK) through the joint funding project CHOICE. This work was further supported by the German Cancer Research Center (DKFZ).

ACKNOWLEDGMENTS

We are grateful to the steady support of German Cancer Research Center (DKFZ) along with the German Cancer Consortium (DKTK). We extend our gratitude to all collaborators and the José Carreras Leukemia Foundation (DJCLS) for supporting this work.

REFERENCES

Abarrategi, A., Foster, K., Hamilton, A., Mian, S. A., Passaro, D., Gribben, J., et al. (2017). Versatile humanized niche model enables study of normal and malignant human hematopoiesis. J. Clin. Invest. 127, 543–548. doi: 10.1172/JCI89364

Abedallah, B. M., Jensen, C. H., Gutierrez, G., Leslie, R. G. Q., Jensen, T. G., and Kassem, M. (2004). Regulation of human skeletal stem cells differentiation by Dlk1/Pref-1. J. Bone Miner. Res. 19, 841–852. doi: 10.1359/JBMR.040118

Adams, G. B., Martin, R. P., Alley, I. R., Chabner, K. T., Cohen, K. S., Calvi, L. M., et al. (2007). Therapeutic targeting of a stem cell niche. Nat. Biotechnol. 25, 238–243. doi: 10.1038/nbt1281

Aguilar-Navarro, A. G., Meza-León, R., Gratzingher, D., Jsárez-Aguilar, F. G., Chang, Q., Ornatsky, O., et al. (2020). Human aging alters the spatial organization between CD34+ hematopoietic cells and adipocytes in bone marrow. Stem Cell Rep. 15, 317–325. doi: 10.1016/j.stemcr.2020.06.011

Ahn, M. B., and Suh, B.-K. (2020). Bone morbidity in pediatric acute lymphoblastic leukemia. Ann. Pediatr. Endocrinol. Metab. 25, 1215–1221. doi: 10.6065/apem.2020.25.1.1

Al-Azab, M., Wang, B., Elkhiider, A., Walana, W., Li, W., Yuan, B., et al. (2020). Indian Hedgehog regulates senescence in bone marrow-derived mesenchymal stem cell through modulation of ROS/mTOR/EBP1, p70S6K1/2 pathway. Aging (Albany N. Y.) 12, 5693–5715. doi: 10.18632/aging.102958
Ambrosi, T. H., Longaker, M. T., and Chan, C. K. F. (2019). A revised perspective of skeletal stem cell biology. Front. Cell Dev. Biol. 7:189. doi: 10.3389/fcell.2019.00189

Antonioli, E., Torres, N., Ferretti, M., Piccinato, C. d. A, and Sertie, A. L. (2019). Chemokines in physiological and pathological bone remodeling. Front. Immunol. 10:2182. doi: 10.3389/fimmu.2019.02182

Cai, J., Qi, H., Yao, K., Yao, Y., Jing, D., Liao, W., et al. (2021). Non-coding RNAs steering the senescence-related progress, properties, and application of mesenchymal stem cells. Front. Cell Dev. Biol. 9:650431. doi: 10.3389/fcell.2021.650431

Calvi, L. M., Adams, G. B., Weibruch, K. W., Weber, J. D., Olson, D. P., Knight, M. C., et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425, 841–846. doi: 10.1038/nature02040

Calvi, L. M., Bramberg, O., Rhee, Y., Weber, J. M., Smith, J. N. P., Basil, M. J., et al. (2012). Osteoblastic expansion induced by parathyroid hormone receptor signaling in murine osteocytes is not sufficient to increase hematopoietic stem cells. Blood 119, 2489–2499. doi: 10.1182/blood-2011-06-360933

Calvi, L. M., Sims, N. A., Hunzelman, J. L., Knight, M. C., Giovannetti, A., Saxton, J. M., et al. (2001). Activated parathyroid hormone/parathyroid hormone-related protein receptor in osteoblastic cells differentially affects cortical and trabecular bone. J. Clin. Invest. 107, 277–286. doi: 10.1172/JCI11296

Casseli, A., Olson, T. S., Otsuru, S., Chen, X., Hofmann, T. J., Nah, H.-D., et al. (2013). IGF-1-mediated osteoblastic niche expansion enhances long-term hematopoietic stem cell engraftment after murine bone marrow transplantation. Stem Cells 31, 2193–2204. doi: 10.1002/stem.1463

Chen, Q., Shou, P., Zheng, C., Jiang, M., Cao, G., Yang, Q., et al. (2016). Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ. 23, 1128–1139. doi: 10.1038/cdd.2015.168

Chulpanova, D. S., Kitaeva, K. V., Tatadzino, L. G., James, V., Rivzanov, A. A., and Solovyeva, V. V. (2018). Application of mesenchymal stem cells for therapeutic agent delivery in anti-tumor treatment. Front. Pharmacol. 9:259. doi: 10.3389/fphar.2018.00259

Costa, L. A., Eiro, N., Fraile, M., Gonzalez, L. O., Saá, J., Garcia-Portabella, P., et al. (2021). Functional heterogeneity of mesenchymal stem cells from natural niches to culture conditions: implications for further clinical uses. Cell. Mol. Life Sci. 78, 447–467. doi: 10.1007/s00018-020-03600-0

Deng, P., Yuan, Q., Cheng, Y., Li, J., Liu, Z., Liu, Y., et al. (2021). Loss of KDM4B exacerbates bone-fat imbalance and mesenchymal stromal cell exhaustion in skeletal aging. Cell Stem Cell 28, 1057.e–1073.e. doi: 10.1016/j.stem.2021.01.010

Ding, L., and Morrison, S. J. (2013). Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. Nature 495, 231–235. doi: 10.1038/nature11885

Dolgalev, I., Tikhonova, A. N. (2021) Connecting the dots: Resolving the bone marrow niche heterogeneity. In Frontiers in cell and developmental biology 8:15. doi: 10.3389/fcell.2021.622519

Ding, L., Saunders, T. L., Enikolopyov, G., and Morrison, S. J. (2012). Endothelial and perivascular cells maintain haematopoietic stem cells. Nature 481, 457–462. doi: 10.1038/nature10783

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8, 315–317. doi: 10.1080/14653240600855905

Dong, L., Yu, W.-M., Zheng, H., Loh, M. L., Bunting, S. T., Pauly, M., et al. (2016). Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. Nature 539, 304–308. doi: 10.1038/nature20131

Duan, C.-W., Shi, J., Chen, J., Wang, B., Yu, Y.-H., Qin, X., et al. (2014). Leukemia propagating cells rebuild an evolving niche in response to therapy. Cancer Cell 25, 778–793. doi: 10.1016/j.cccr.2014.04.015

Duarte, D., Hawkins, E. D., Akinduro, O., Ang, H., de Filippo, K., Kong, I. Y., et al. (2018). Inhibition of endosteal vascular niche remodeling rescues hematopoietic stem cell loss in AML. Cell Stem Cell 22, 64.e–77.e. doi: 10.1016/j.stem.2017.11.006

Fan, Y., Hanai, J.-I., Le, P. T., Bi, R., Maridas, D., DeMambro, V., et al. (2017). Parathyroid hormone directs bone marrow mesenchymal cell fate. Cell Metab. 26, 661–672. doi: 10.1016/j.cmet.2017.01.001

Fathi, E., Sanaa, Z., and Farahzadi, R. (2019). Mesenchymal stem cells in acute myeloid leukemia: a focus on mechanisms involved and therapeutic concepts. Blood Res. 54, 165–174. doi: 10.5045/br.2019.54.3.165
Helbling, P. M., Piñeiro-Yáñez, E., Gerosa, R., Boettcher, S., Al-Shahrour, F., Halabian, R., Tehrani, H. A., Jahanian-Najafabadi, A., and Habibi Roudkenar, Guo, H., Jin, D., Zhang, Y., Wright, W., Bazuine, M., Brockman, D. A., et al. (2010). Organic acid tumor stromal microenvironment and preferentially expands short-term hematopoietic stem cells. Blood 114, 4054–4063. doi: 10.1182/blood-2009-03-205823
Fulzele, K., Krause, D. S., Panaroni, C., Saini, V., Bruckner, B., and Jessop, H. L., Noble, B. S., and Cryer, A. (1994). The differentiation of a potential mesenchymal stromal cell population within ovine bone marrow. Cytotherapy 15, 365–376. doi: 10.1016/j.cytog.2016.02.0039
Frisch, B. J., Hoffman, C. M., Latchney, S. E., LaMere, M. W., Myers, J., Ashton, J., et al. (2019). Aged marrow macrophages expand platelet-biased hematopoietic stem cells via Interleukin1B. JCI Insight 5(6):124213. doi: 10.1172/jci.insight.124213
Frisch, B. J., Porter, R. L., Gigliotti, B. J., O’Keefe, R. J., et al. (2009). In vivo prostaglandin E2 treatment alters the bone marrow microenvironment and preferentially expands short-term hematopoietic stem cells. Blood 114, 4054–4063. doi: 10.1182/blood-2009-03-205823
Geyh, S., Ozt, S., Cadeddu, R.-P., Fröbel, J., Brückner, B., and Kündgen, A., et al. (2013). Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia 27, 1841–1851. doi: 10.1038/leu.2013.193
Geyh, S., Rodríguez-Paredes, M., Jäger, P., Khandanpour, C., Cadeddu, R.-P., Gutekunst, J., et al. (2016). Functional inhibition of mesenchymal stromal cells in acute myeloid leukemia. Leukemia 30, 683–691. doi: 10.1038/leu.2015.325
Ghazanfari, R., Zacharaki, D., Li, H., Ching Lim, H., Soneji, S., and Scheding, S. (2017). Human primary bone marrow mesenchymal stromal cells and their role in tissue engineering: a review. J. Biomed. Nanotechnol. 13, 175–189. doi: 10.1016/j.nanotechnol.2019.07.006
Guo, H., Jin, D., Zhang, Y., Wright, W., Bazuine, M., Brockman, D. A., et al. (2010). Lipocalin-2 deficiency impairs thermogenesis and potentiates diet-induced insulin resistance in mice. Diabetes 59, 1376–1385. doi: 10.2337/db09-20643
Garrido, S. M., Appelbaum, F. R., Willman, C. L., and Banker, D. E. (2001). Acute leukemia. N. Engl. J. Med. 345, 1841–1851. doi: 10.1038/35061094
Geyh, S., Ozt, S., Cadeddu, R.-P., Fröbel, J., Brückner, B., Kündig, A., et al. (2013). Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia 27, 1841–1851. doi: 10.1038/leu.2013.193
Geyh, S., Rodríguez-Paredes, M., Jäger, P., Khandanpour, C., Cadeddu, R.-P., Gutekunst, J., et al. (2016). Functional inhibition of mesenchymal stromal cells in acute myeloid leukemia. Leukemia 30, 683–691. doi: 10.1038/leu.2015.325
Kumar, B., Garcia, M., Weng, L., Jung, X., Murakami, J. L., Hu, X., et al. (2018). Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. *Leukemia* 32, 575–587. doi: 10.1038/leu.2017.259

Kumar, S., and Geiger, H. (2017). HSC niche biology and HSC expansion Ex Vivo. *Trends Mol. Med.* 23, 799–819. doi: 10.1016/j.trendsmd.2017.07.003

Kunisaki, Y., Bruns, I., Scheiermann, C., Ahmed, J., Pinho, S., Zhang, D., et al. (2013). Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature* 502, 637–643. doi: 10.1038/nature12612

Kuznetsova, S. A., Riminucci, M., Zirani, N., Tsutsui, T. W., Corsi, A., Calvi, L., et al. (2004). The interplay of osteogenesis and hematopoiesis: expression of a constitutively active PTH/PTHrP receptor in osteogenic cells perturbs the establishment of hematopoiesis in bone and of skeletal stem cells in the bone marrow. *J. Cell Biol.* 167, 1113–1122. doi: 10.1083/jcb.200408079

Lawal, R. A., Zhou, X., Batey, K., Hoffman, C. M., Georgey, M. A., Radlke, F., et al. (2017). The notch ligand Jagged1 regulates the osteostemline by maintaining the osteoprogenitor pool. *J. Bone Miner. Res.* 32, 1320–1331. doi: 10.1002/jbmr.3106

Lee, M. W., Ryu, S., Kim, D. S., Lee, J. W., Sung, K. W., Koo, H. H., et al. (2019). Mesenchymal stem cells in suppression or progression of hematologic malignancy: current status and challenges. *Leukemia* 33, 597–611. doi: 10.1038/s41375-018-0373-9

Leimkühler, N. B., Gleitz, H. F. E., Ronghui, L., Snoeren, I. A. M., Fuchs, S. N. R., Lee, M. W., Ryu, S., Kim, D. S., Lee, J. W., Sung, K. W., Koo, H. H., et al. (2018). Senescent mesenchymal stem/stromal cells and restoring their cellular functions. *World J. Stem Cells* 12, 966–985. doi: 10.4252/wjsc.v12.i9.966

Leyv, O., Kuai, R., Siren, E. M. J., Bhere, D., Milton, Y., Nissar, N., et al. (2020). Adrenergic nerve degeneration in bone marrow drives aging of the hematopoietic stem cell niche. *Nat. Med.* 26, 782–791. doi: 10.1038/s41596-018-0030-x

Matsuzaki, Y., Masuchi, Y., and Okano, H. (2014). Leptin receptor makes its mark on MSCs. *Cell Stem Cell* 15, 112–114. doi: 10.1016/j.stem.2014.07.001

Medyouth, M., Mossier, M., Jann, J.-C., Nolte, F., Raffel, S., Herrmann, C., et al. (2014). Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell Stem Cell* 14, 824–837. doi: 10.1016/j.stem.2014.02.020

Méndez-Ferrer, S., Bonnet, D., Steensma, D. P., Gribben, J. R., Ghobrial, I. M., Gribben, J. G., et al. (2020). Bone marrow niches in haematological malignancies. *Nat. Rev. Cancer* 20, 285–298. doi: 10.1038/s41568-020-0245-2

Méndez-Ferrer, S., Michurina, T. V., Ferraro, F., Mazzoom, A. R., MacArthur, B. D., Lira, S. A., et al. (2010). Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* 466, 829–834. doi: 10.1038/nature09262

Meng, Q.-S., Liu, J., Wei, L., Fan, H.-M., Zhou, X.-H., and Liang, X.-T. (2020). Senescent mesenchymal stem/stromal cells and restoring their cellular functions. *World J. Stem Cells* 12, 966–985. doi: 10.4252/wjsc.v12.i9.966

Meza-León, B., Graftinger, D., Aguilar-Nava, A. G., Juárez-Aguilar, F. G., Rebel, V. I., Tolrakovic, E., et al. (2021). Human, mouse and dog bone marrow show similar mesenchymal stromal cells within a distinctive microenvironment. *Exp. Hematol.* 10.1016/j.exphem.2021.06.006 vol page.

Mian, S. A., Abarrategi, A., Kong, K. L., Rouault-Pierre, K., Wood, H., Oecdovken, C. A., et al. (2021). Ectopic humanized mesenchymal niche in mice enables robust engraftment of myelodysplastic stem cells. *Blood Cancer Discov.* 2, 135–145. doi: 10.1182/bloodcancerjournals.2020-0161

Michelis, F. V., Hedley, D. W., Malhotra, S., Chow, S., Loach, D., Gupta, V., et al. (2019). Mobilization of leukemic cells using perilxifor as part of a myeloablative preparative regimen for patients with acute myelogenous leukemia undergoing allografting: assessment of safety and tolerability. *Biol. Blood Marrow Transplant.* 25, 1158–1163. doi: 10.1016/j.bbmt.2019.01.014

Mihara, K., Hiroyama, T., Sudo, K., Danjo, L., Nagasawa, T., and Nakamura, Y. (2008). Lipocalin-2-mediated growth suppression is evident in human erythroid and monocytic/macrophage lineage cells. *J. Cell. Physiol.* 215, 526–537. doi: 10.1002/jcp.21033

Moll, G., Hoogduijn, M. J., and Ankrum, J. A. (2020). Editorial: safety, efficacy and mechanisms of action of mesenchymal stem cell therapies. *Front. Immunol.* 11:243. doi: 10.3389/fimmu.2020.00243

Morikawa, S., Naka, Y., Kuroki, Y., Tazawa, M., Nomura, M., Hiraoka, K., Hara, Y., et al. (2009). Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. *J. Exp. Med.* 206, 2483–2496. doi: 10.1084/jem.20091046

Mosialou, I., Shiokh, S., Luo, X., Petrooulou, P. I., Pansati, K., Biskiriska, B., et al. (2020). Lipocalin-2 counteracts metabolic dysregulation in obesity and diabetes. *J. Exp. Med.* 217:e20191261. doi: 10.1084/jem.20191261

Nakahara, F., Borger, D. K., Wei, Q., Pinho, S., Maryanovich, M., Zakhala, A. H., et al. (2019). Engineering a haematopoietic stem cell niche by revatilizing...
mesenchymal stromal cells. Nat. Cell. Biol. 21, 560–567. doi: 10.1038/s41556-019-0308-3
Oh, J. H., Karadeniz, F., Seo, Y., and Kong, C.-S. (2020). Effect of quercetin 3-O-β-D-galactopyranoside on the adipogenic and osteoblastogenic differentiation of human bone marrow-derived mesenchymal stromal cells. Int. J. Mol. Sci. 21, 8044. doi: 10.3390/ijms21128044
Okumura, T., Wang, S. S. W., Takahashi, S., Tu, S. P., Ng, V., Erickson, R. E., et al. (2009). Identification of a bone marrow-derived mesenchymal progenitor cell subset that can contribute to the gastric epithelium. Lab. Invest. 89, 1410–1422. doi: 10.1038/labinvest.2009.88
Osagie-Clouard, L., Sanghani, A., Coathup, M., Briggs, T., Bostrom, M., and Blunn, G. (2017). Parathyroid hormone 1-34 and skeletal anabolic action: the use of parathyroid hormone in bone formation. Bone Joint Res. 6, 14–21. doi: 10.1002/bjr.20356
Pan, J.-X., Xiong, L., Zhao, K., Zeng, P., Wang, B., Tang, F.-L., et al. (2018). YAP promotes osteogenesis and suppresses adipogenic differentiation by regulating β-catenin signaling. Bone research 6:18. doi: 10.1038/s41421-018-0018-7
Pinho, S., and Frenette, P. S. (2019). Haematopoietic stem cell activity and interactions with the niche. Nat. Rev. Mol. Cell Biol. 20, 303–320. doi: 10.1038/s41580-019-0103-9
Pinho, S., Lacombe, J., Hanoun, M., Mizoguchi, T., Bruns, I., Kunisaki, Y., et al. (2019). mTOR as a central regulator of lifespan and aging. Cell Stem Cell 25, 2138–2147. doi: 10.1016/j.stem.2019.06.008
Qin, P., Fink, P. Y., Hou, W., Fu, R., Zhang, Y., Wang, X., et al. (2021). Integrated microRNA and miRNA dysregulation in mesenchymal stem cells from myelodysplastic syndrome and acute myeloblastic leukemia. Leuk. Res. 63, 62–71. doi: 10.1016/j.leukres.2017.10.006
Rafii, S., and Fontana, A. (2019). A hematopoietic microenvironment. Cell 179, 1351–1367. doi: 10.1016/j.cell.2019-03-083
Roboz, G. J., Ritchie, E. K., Dault, Y., Lam, L., Marshall, D. C., Cruz, N. M., et al. (2018). Gli1+ mesenchymal stromal cells are a key driver of bone marrow fibrosis and an important cellular therapeutic target. Cell Stem Cell 22, 785.e–800.e. doi: 10.1016/j.stem.2017.03.008
Shi, S., Zhang, Q., Xia, Y., You, B., Shan, Y., Bao, L., et al. (2016). Mesenchymal stem cell-derived exosomes facilitate nasopharyngeal carcinoma progression. Am. J. Cancer Res. 6, 459–472.
Shi, W., Xu, C., Gong, Y., Wang, J., Ren, Q., Yan, Z., et al. (2021). Rhoa/Rock activation represents a new mechanism for inactivating Wnt/β-catenin signaling in the aging-associated bone loss. Cell Regen. 10:8. doi: 10.1186/s13169-020-00071-3
Siegel, K. R., Mullally, A., Doguord, A., Peisker, F., Hoogenboezem, R., van Strien, P. M. H., et al. (2017). Gli1+ mesenchymal stromal cells are a key driver of bone marrow fibrosis and an important cellular therapeutic target. Cell Stem Cell 20, 785.e–800.e. doi: 10.1016/j.stem.2017.03.008
Stuempfle, K. P., Du, X., Imanishi, H., Kuniyuki, Y., Semba, Y., Noble, T., et al. (2020). Transforming growth factor beta induced cell cycle arrest of human hematopoietic cells requires p57KIP2 up-regulation. Proc. Natl. Acad. Sci. U.S.A. 101, 15231–15236.
doi: 10.1073/pnas.0406771101
Stumpf, P. S., Du, X., Imanishi, H., Kuniyuki, Y., Semba, Y., Noble, T., et al. (2020). Transforming growth factor beta-induced cell cycle arrest of human hematopoietic cells requires p57kip2 up-regulation. Proc. Natl. Acad. Sci. U.S.A. 101, 15231–15236.
doi: 10.1073/pnas.0406771101
Stumpf, P. S., Du, X., Imanishi, H., Kuniyuki, Y., Semba, Y., Noble, T., et al. (2020). Transforming growth factor beta-induced cell cycle arrest of human hematopoietic cells requires p57KIP2 up-regulation. Proc. Natl. Acad. Sci. U.S.A. 101, 15231–15236.
Xia, W., Zhang, F., Xie, C., Jiang, M., and Hou, M. (2015). Macrophage migration
Xie, Y., Yin, T., Wiegraebe, W., He, X. C., Miller, D., Stark, D., et al. (2009). Lipocalin 2 regulates brown fat activation via a nonadrenergic activation mechanism. J. Biol. Chem. 285, 9777ñ9786. doi: 10.1074/jbc.M109.034776

Yao, H., Miura, Y., Yoshioka, S., Miura, M., Hayashi, Y., Tamura, A., et al. (2014). Parathyroid hormone enhances hematopoietic expansion via upregulation of caderhin-11 in bone marrow mesenchymal stromal cells. Stem Cells Dev 23, 2255ñ2265. doi: 10.1002/stem.1701

Yoon, K.-A., Cho, H.-S., Shin, H.-I., and Cho, J.-Y. (2012). Differential regulation of CXCL5 by FGFR2 in osteoblastic and endothelial niche cells supports hematopoietic stem cell migration. Stem Cells Dev 21, 3391ñ3402. doi: 10.1089/scd.2012.0128

Yoon, K.-A., Son, Y., Choi, Y.-J., Kim, J.-H., and Cho, J.-Y. (2017). Fibroblast growth factor 2 supports osteoblastic niche cells during hematopoietic homeostasis recovery after bone marrow suppression. Cell Commun. Signal. 15:25. doi: 10.1186/s41994-017-0181-2

Yoshihara, H., Arai, F., Hosokawa, K., Hagiwara, T., Takubö, K., Nakamura, Y., et al. (2007). Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell Stem Cell 1, 685ñ697. doi: 10.1016/j.stem.2007.10.020

Yu, B., Zhao, X., Yang, C., Crane, J., Xian, L., Lu, W., et al. (2012). Parathyroid hormone induces differentiation of mesenchymal stromal/stem cells by enhancing bone morphogenetic protein signaling. J. Bone Miner. Res. 27, 2001ñ2014. doi: 10.1002/jbmr.1663

Yu, V. W. C., Lymerpi, O., Oki, T., Jones, A., Swiatek, P., Vasic, R., et al. (2016). Distinctive mesenchymal-par enchymal cell pairings govern B cell differentiation in the bone marrow. Stem Cell Rep. 7, 220ñ235. doi: 10.1016/j.stemcr.2016.06.009

Zambetti, N. A., Ping, Z., Chen, S., Kenswil, K. J. G., Mylona, M. A., Sanders, M. A., et al. (2016). Mesenchymal inflammation drives genotoxic stress in hematopoietic stem cells and predicts disease evolution in human pre-leukemia. Cell Stem Cell 19, 613ñ627. doi: 10.1016/j.stem.2016.08.021

Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.-G., et al. (2003). Identification of the haematopoietic stem cell niche and control of the niche size. Nature 425, 836ñ841. doi: 10.1038/nature02041

Zhang, Y., Guo, H., Deis, J. A., Mashek, M. G., Zhao, M., Ariyakumar, D., et al. (2014). Lipocalin 2 regulates brown fat activation via a nonadrenergic activation mechanism. J. Biol. Chem. 289, 22063ñ22077. doi: 10.1074/jbc.M114.559104

Zhang, Y., Patel, S., Abdelouahab, H., Wittner, M., Willekens, C., Shen, S., et al. (2012). CXCR4 inhibitors selectively eliminate CXCR4-expressing human acute myeloid leukemia cells in NOG mouse model. Cell Death Dis. 3:e396. doi: 10.1038/cddis.2012.137

Zheng, X., Wang, Q., Xie, Z., and Li, J. (2021). The elevated level of IL-1β in the bone marrow of aged mice leads to MSC senescence partly by down-regulating Bmi-1. Exp. Gerontol. 148:111313. doi: 10.1016/j.exger.2021.11.1313

Zhou, L., Yao, L., Tower, R. J., Wei, Y., Miao, Z., Park, J., et al. (2020). Single cell transcriptionomics identifies a unique adipose lineage cell population that regulates bone marrow environment. Elife 9:e54695. doi: 10.7554/eLife.54695

Zhou, W., Lin, J., Zhao, K., Jin, K., He, Q., Hu, Y., et al. (2019). Single-cell profiles and clinically useful properties of human mesenchymal stem cells of adipose and bone marrow origin. Am. J. Sports Med. 47, 1722ñ1733. doi: 10.1177/0363546519848678

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Woods and Guezguez. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.