Published in final edited form as:

Bioessays. 2018 March ; 40(3): . doi:10.1002/bies.201700190.

Bone marrow microenvironment in normal and deranged hematopoiesis: Opportunities for regenerative medicine and therapies

Shawn M. Sarkaria*, Matthew Decker*, and Lei Ding¹

Department of Rehabilitation and Regenerative Medicine, Department of Microbiology and Immunology, Columbia Stem Cell Initiative, Columbia University Medical Center, New York, NY, 10032, USA

Abstract

Various cell types cooperate to create a highly organized and dynamic microenvironmental niche in the bone marrow. Over the past several years, the field has increasingly recognized the critical roles of the interplay between bone marrow environment and hematopoietic cells in normal and deranged hematopoiesis. These advances rely on several new technologies that have allowed us to characterize the identity and roles of these niches in great detail. Here, we review the progress of the last several years, list some of the outstanding questions in the field and propose ways to target the diseased environment to better treat hematologic diseases. Understanding the extrinsic regulation by the niche will help boost hematopoiesis for regenerative medicine. Based on natural development of hematologic malignancies, we propose that combinatory targeting the niche and hematopoietic intrinsic mechanisms in early stages of hematopoietic malignancies may help eliminate minimal residual disease and have the highest efficacy.

Graphical abstract

The bone marrow is the home for blood-forming hematopoietic stem and progenitor cells. Normal generation of blood and immune cells depends on highly regulated interactions between these stem and progenitor cells with their environment. Emerging evidence suggests that aiming at the interaction mechanisms is a key to boost hematopoiesis and eradicate malignant hematopoietic cells.

¹Author for correspondence: Department of Rehabilitation and Regenerative Medicine, Columbia University Medical Center, 630 West 168th ST, P&S 7-513, New York, NY, 10032; phone 212-305-7468; fax 212-342-3889; ld2567@cumc.columbia.edu.

*these authors contribute equally.

Keywords

Hematopoiesis; HSC; niche; hematological diseases; cross-talk; premalignant; treatment

1. Introduction

In mammals, many somatic cells have a definite life span that is significantly shorter than the life span of the organism. For example, in the hematopoietic system, red blood cells typically live for about 120 days¹. Tissue resident stem cells solve the problem of somatic cell turnover. These stem cells can differentiate into all cell types within a given tissue. They can also maintain a stable pool of stem cells by self-renewal divisions, where one or two stem cells are generated following each cell division. Stem cells must carefully balance differentiation and self-renewal activity to achieve tissue homeostasis: too much differentiation or too little self-renewal depletes stem cells while too little differentiation or too much uncontrolled self-renewal often leads to cancer. Within a given tissue, stem cells receive instructional cues from a specialized microenvironment, called the niche. Understanding how the niche maintains and regulates stem cells offers great opportunity to harness the power of stem cells for regenerative medicine.

Hematopoiesis is the physiologic process of generating all blood and immune cells. It has served as the paradigm for studying stem cell biology. The idea of a somatic stem cell was first proposed and proven in the hematopoietic system². By definition, a hematopoietic stem cell (HSC) is a cell that can repopulate all blood cell lineages when transplanted into recipients. As such, HSC transplantation remains a mainstay of clinical treatment for hematologic diseases. While recent evidence suggests that restricted hematopoietic progenitors may generate most of the mature blood cells at steady state^{3, 4}, HSCs need to be maintained throughout life to replenish downstream progenitors and to regenerate the whole hematopoietic system after stress. With the help of advances in methods, such as new markers to identify HSCs and their corresponding niche cells, novel imaging technologies, and precise functional genetics, the field has seen significant advancement in the past ten

years. We have defined the cellular components of the bone marrow HSC niche. Osteoblasts were initially proposed to be the key component of the niche⁵. However, improved HSC markers and imaging techniques revealed that most *bona fide* HSCs actually reside in a perivascular niche rather than an osteoblastic niche in mice and zebrafish⁶⁻¹². Accumulating functional studies are lending more weight to the critical roles of the bone marrow perivascular niche in maintaining HSCs⁵. Given the essential role of the niche in maintaining normal hematopoiesis, it is not surprising that researchers have begun to pursue detailed studies examining the role of the niche in hematopoietic diseases. These discoveries have introduced exciting new therapeutic opportunities that have yet to reach their full potential in the clinic. Here, we will review the evolving landscape of hematopoietic niche research with an emphasis on studies within the past five years, highlight some of the outstanding questions in the field and propose how to use the knowledge we have to better design rational therapeutics.

2. The niche for normal hematopoiesis

2.1. New tools help answer old questions

The bone marrow houses many cell types, including hematopoiesis-supporting stromal cells. These cells co-exist in harmony to maintain efficient and balanced hematopoiesis *in vivo* (Figure 1). Early studies of the stromal system in the bone marrow required *ex vivo* manipulation; cells were harvested and subdivided based on their physical properties or cell surface antigen profile¹³. Insights from this foundational work led to the development of marker combinations and genetic tools that in combination with new imaging techniques^{10, 13-15} allowed detailed analysis of these labeled cells on bone marrow sections. Finally, the increased number and availability of conditional Cre-recombinase mouse strains¹³ made it possible to genetically manipulate almost any bone marrow population *in vivo*. Below we describe how recent studies have utilized these advances to characterize the cellular components of the bone marrow microenvironment.

2.2. Where do HSCs reside?

HSCs reside at the apex of the hematopoietic hierarchy; they are the only blood cell type that can provide long-term multilineage reconstitution. During homeostasis, adult HSCs primarily reside in the bone marrow. Revealing the microenvironment that maintains HSCs requires the precise localization of HSCs *in situ*. Although decades of work based on flow cytometry and transplantation assays has identified markers that can be used to sort HSCs to near single-cell purity, these markers are not suitable for staining HSCs on tissue sections. It was the discovery of two-color CD150+CD48-LIN-CD41- staining that allowed direct visualization of HSCs in their native microenvironment⁷.

Initial surveys of thinly sliced bone marrow sections using SLAM markers showed HSCs in close proximity to bone marrow sinusoids⁷. It was realized that the HSC population defined by these markers is heterogeneous, the quiescent ones having the greatest reconstitution potential¹⁶. Interestingly, it was proposed that quiescent HSCs are closely associated with arterioles but not sinusoids¹⁷. However, recent studies utilizing other new single-color genetic markers did not support these findings. Like the CD150+LIN-CD48-CD41-

population, genetically labeled Hoxb5⁺¹¹, α -catulin⁺¹⁸ or Map17⁺¹² cells can reconstitute all blood cell lineages in lethally irradiated recipients after transplantation of between one to five cells. All three marker sets define the HSC population at a very high level of purity, both quiescent and dividing ones. 3-D computerized reconstruction of bone marrow sections showed Hoxb5⁺ cells are almost uniformly in contact with vascular VEcadherin⁺ endothelial cells¹¹. Clearing and deep optical sectioning of long bones from α -catulin⁺ mice found that quiescent and non-quiescent HSCs overwhelmingly reside in the central marrow near sinusoidal blood vessels and leptin-receptor positive (LepR⁺) mesenchymal stromal cells¹⁰. Similarly, Map17⁺ primitive HSCs localize close to sinusoidal vessels¹². Thus, overwhelming data suggest that bone marrow HSCs are perisinusoidal, which has been extensively reviewed elsewhere^{5, 19, 20}. Further studies are needed to reveal the significance of the arteriolar localization of HSCs.

2.3. Hematopoietic progenitor cells also need a home

Most HSC markers are defined based on transplantation assays, where candidate cells are transplanted into irradiated recipients and assessed for their ability to provide long-term multilineage reconstitution. Surprisingly, recent *in vivo* tracking of endogenous hematopoiesis in non-transplanted mice discovered that hematopoietic progenitors but not HSCs are directly responsible for the bulk of steady state hematopoiesis^{3, 4, 21}. However, another HSC lineage-tracing study reported that about 60% of steady state hematopoiesis is from HSCs¹². Clearly, more work is required to resolve this discrepancy, but these studies raised an interesting question regarding how progenitors are regulated in the bone marrow environment *in vivo*. Hematopoietic progenitor cells are distinguished from HSCs by a restriction of lineage potential and/or diminished capacity for long-term self-renewal. Included in this definition are committed progenitors such as common myeloid and common lymphoid progenitors (CMPs and CLPs), granulocyte and monocyte progenitors (GMPs), and multipotent progenitors (MPPs).

MPPs can further be divided into distinct populations based cell surface antigen expression^{22, 23}. These progenitor subgroups have distinct molecular profiles and lineages biases^{24, 25}. Notably, in damaged and diseased conditions, myeloid-biased MPPs expand and drive a majority of blood cell production²⁵. A similar expansion is seen in downstream committed GMPs after myeloablative stress in a localized fashion²⁶. These shifts are accompanied by a decrease in HSC self-renewal, suggesting that HSCs increase their rate of asymmetric cell division to generate more myeloid-biased progenitors in stress hematopoiesis.

The distinct functional properties of HSCs from other hematopoietic progenitors suggest distinct niches for hematopoietic progenitors. Indeed, a subset of lymphoid progenitors appears to localize close to the bone surface²⁷. Most stages of B cell development are dependent on the perivascular niches^{28, 29}. It is not clear what cells create niches for MPPs and GMPs although GMP expansion depends on factors from the vasculature, such as SCF²⁶. As certain AMLs are proposed to arise from GMP-like leukemia initiating cells (LICs)³⁰, it is of great importance to understand how GMPs are interacting with the bone marrow niche. Moving forward, elucidating the components of niches of distinct progenitors will

help reveal the mechanisms of their regulation both under steady state and in diseased conditions.

2.4. Bone marrow mesenchymal cells: critical regulators of hematopoiesis

The bone marrow stroma is a mixture of mesenchymal stem and progenitor cells and their progenies that includes adipocytes, osteolineage cells, pericytes, chondrocytes, and fibroblasts. Early studies found that manipulation of osteolineage populations appeared to have an effect on hematopoiesis both *in vitro* and *in vivo*³¹⁻³³. However, experimentally ablating or expanding large numbers of mesenchymal cells *in vivo* may have cascading indirect effects on other bone marrow populations. More sophisticated genetic tools were thus needed to identify the mesenchymal cells that were directly regulating hematopoiesis.

Identifying niche cells that generate essential HSC maintenance factors is an effective way to uncover the key component of the niche. Few cytokines are known genetically required for HSC maintenance, including SCF, CXCL12 and TPO. Utilizing genetic reporter mice, it was discovered that LepR⁺ mesenchymal stromal cells expressed high levels of key HSC niche factors SCF and CXCL12^{27, 34}. LepR⁺ cells significantly overlap with a population of adipo-osteogenic progenitors - CXCL12-abundant reticular (CAR) cells – that have been shown to regulate HSCs and hematopoietic progenitors³⁵. LepR⁺ cells also express low levels of the Nestin-GFP transgene³⁶ but not endogenous Nestin or other Nestin transgenes³⁴. Transgene-associated distinct genomic integration counts for the inconsistent expression of different Nestin markers^{37, 38}. This contributes to some confusion in the field when antibodies specific for endogenous Nestin were used as markers for bone marrow niche cells. Cautions need to be taken when using Nestin as a marker for bone marrow HSC niche cells.

Deletion of *Scf* and *Cxcl12* from LepR-Cre⁺ stromal cells greatly depleted HSCs and perturbed hematopoiesis. The LepR-Cre lineage cell population is enriched for mesenchymal stem and progenitor activity³⁶. Studies with additional reporter and Cre-lineage lines confirmed the connection between mesenchymal progenitors and support of hematopoiesis³⁹⁻⁴¹. Consistent with a key role of mesenchymal stromal cells, conditional deletion of *Foxc1* from LepR⁺ cells depleted HSCs in the bone marrow⁴². Mesenchymal progenitor populations also give lineage instruction to hematopoietic progenitors and maintain B cell lymphopoiesis through CXCL12 and IL-7 signaling^{27, 29}.

Fully differentiated mesenchymal cells govern hematopoiesis through a variety of mechanisms. Osteoblasts contribute to lymphoid progenitor maintenance²⁷, erythropoiesis⁴³, and megakaryopoiesis⁴⁴. Bone-embedded osteocytes regulate myelopoiesis via G-CSF signaling^{45, 46}. After irradiation, adipocytes can transiently support low numbers of HSCs through SCF secretion⁴⁷. However, persistent bone marrow adipogenesis – as seen in aging and disease – may lead to hematopoietic dysfunction^{42, 48}. Thus, robust hematopoiesis depends on the balance between progenitors, osteolineage, and adipolineage cells in the bone marrow stroma.

2.5. Hematopoiesis depends on bone marrow vasculature

The bone marrow vasculature serves the standard physiological functions of oxygenation and blood cell transport, but also has specialized roles in homeostatic and regenerative hematopoiesis. Vascular endothelial cells drive self-renewal and trafficking behavior in HSCs through a cocktail of signaling and chemotactic modalities like SCF³⁴, CXCL12²⁷, Notch⁴⁹, ROBO4⁵⁰, pleiotrophin⁵¹ and EPHB4⁵². The bone marrow vasculature also indirectly regulates hematopoiesis through developmental crosstalk with osteolineage mesenchymal cells^{53, 54}.

After irradiation, vascular regeneration is essential to hematopoietic recovery^{55, 56}. This effect is dependent on endothelial production of Notch ligands, EGF, and other angiocrine signals⁵⁷⁻⁶⁰, which in part act by repressing NF- κ B inflammatory signaling in the bone marrow microenvironment^{61, 62}. These same pathways may play a role in aging and disease; boosting endothelial Notch signaling in aged mice increases the functionality of hematopoietic stem and progenitor cells⁴⁹. A parallel line of evidence comes from A-ZIP/FI 'fatless' mice, which recover their blood vessels much more quickly and show enhanced hematopoietic function post-radioablation^{47, 63}.

2.6. Cross-talk between organs influences hematopoiesis

Hematopoiesis can be perturbed or maintained by inputs that arise from outside the bone marrow. For example, the body's nervous system modulates hematopoiesis through several distinct mechanisms. Circadian cycles originating in the suprachiasmatic nucleus modulate HSC trafficking via adrenergic signaling to bone marrow CXCL12-secreting stromal cells^{64, 65}. The central nervous system also influences hematopoietic development and HSC mobilization through cholinergic stimulation of the hypothalamic-pituitary-adrenal axis^{66, 67}. Finally, non-myelinating Schwann cells of the peripheral nervous system locally secrete TGF- β that maintains HSC quiescence⁶⁸.

Physiological states, nutritional intake, and endocrine signals all influence the hematopoietic steady state. Levels of freely diffusing oxygen⁶⁹ and nitric oxide⁷⁰ appear to have major effects on hematopoietic stem and progenitor cells. However, the effects of oxygen are likely mediated through indirect mechanisms since deletion of core hypoxia factors HIF1 α and HIF2 α from hematopoietic cells has no effect on steady state hematopoiesis^{71, 72}. Calorie restriction or the absence of key dietary nutrients like valine can greatly perturb native hematopoiesis^{73, 74}. Recent evidence suggests that changes to gut microbiota populations may in turn alter the bone marrow microenvironment^{75, 76}. Finally, circulating estrogen⁷⁷ and erythropoietin⁷⁸ directly regulate erythrocyte production by signaling to hematopoietic stem and progenitor cells.

2.7. Mature hematopoietic cells dictate HSC behavior

Hematopoietic stem and progenitor cells are directly responsible for blood cell production. However, differentiated hematopoietic lineage cells play important roles in both steady state and stress hematopoiesis by regulating HSC and progenitors. Megakaryocytes secrete TGF β -1 and CXCL4 to maintain HSC quiescence, but drive HSC expansion through FGF-1 after chemotherapeutic challenge^{79, 80}. Similarly, macrophages appear to play a role in HSC

dormancy^{81, 82} and regulate erythropoiesis in homeostasis and disease states⁸³. Foxp3+ regulatory T cells protect HSCs from immune surveillance⁸⁴ and support B-cell lymphopoiesis in stress conditions⁸⁵. Together with the stromal and systemic factors, these hematopoietic cell-derived signals maintain balanced renewal and differentiation of the hematopoietic system.

2.8. Hematopoietic niches may have a conserved structure

Biological motifs found in the bone marrow microenvironment are recapitulated in other hematopoietic organs. In the spleens of mice with induced extramedullary hematopoiesis, HSCs are found localized in perisinusoidal niches near SCF and CXCL12-secreting TCF21+ perivascular stromal cells⁸⁶. The fetal liver HSC niche appears to be closely associated with the periportal vasculature⁸⁷. The lung contains a permanent population of hematopoietic progenitor cells in perivascular regions near the pulmonary capillary beds⁸⁸. In total this suggests a conserved regulatory relationship between hematopoietic stem and progenitor cells, mesenchymal stroma, and vasculature.

2.9. Hematopoiesis is altered by aging bone marrow vasculature

In old age, the hematopoietic system shows greater myeloid bias and diminished HSC function. These changes have historically been ascribed, at least in part, to increased adipogenesis and decreased osteogenesis in the aged mesenchymal population⁸⁹. However, it was recently discovered that adipocytes support hematopoietic recovery through SCF signaling⁴⁷. Interestingly, aged HSC function is rejuvenated by activated endothelial Notch signaling⁴⁹. These studies suggest that bone marrow vasculature, not adipocytes, may drive the aging phenotype of hematopoiesis. More studies are needed to carefully elucidate the role of the bone marrow niche in ageing.

3. Deranged hematopoiesis

3.1. Hematopoietic diseases: interplay between seed and soil

Greater understanding of the cellular constituents and key supportive factors required for physiologic bone marrow niche function has created a new paradigm for thinking about hematologic disease. Instead of a hematopoietic cell-centric view, where cell-intrinsic alterations are the focus of investigation, a more complete picture of interdependence between blood cells and surrounding stromal cells has emerged. Malignancy serves as a good example because it is known that HSPCs acquire mutations in a stepwise manner⁹⁰, and the niche can exert negative or positive selective pressure depending on a variety of environmental factors. A precarious balance is maintained until the delinquent cells become unhinged from niche regulation, losing control of highly evolved growth and differentiation decisions. This transition point likely distinguishes subclinical from clinical disease. By dissecting the molecular and cellular mechanisms that control such changes, niche research promises to improve prevention and treatment of hematologic diseases (Figure 2).

3.2. Aberrant Niche cells can initiate hematologic disease

The idea of an abnormal bone marrow microenvironment giving rise to disease dates back to at least the 1970s when Knospe and Crosby proposed that aplastic anemia (AA), a misnomer

for bone marrow aplasia, may result from immune-mediated destruction of the sinusoidal microcirculation⁹¹. Their experimental observations were significant because they recognized that developing hematopoietic cells depend on an infrastructure of other cell types for survival and function, but also because they reasoned that blood cell disorders can derive from cell extrinsic pathology. Subsequent data have supported the role of autoimmunity in AA pathogenesis, and immunosuppressive therapy has markedly improved outcomes for these patients⁹². A similar concept of niche-induced disease has emerged over the last decade largely based on studies performed in genetically altered murine models, some of which are highlighted below (comprehensively reviewed elsewhere^{93, 94}). Osteoprogenitor cells with deletion of *Dicer1*, a microRNA-processing gene, downregulate the ribosomal assembly protein *Sbds* leading to myelodysplasia and sporadic transformation to acute myeloid leukemia (AML)⁹⁵. Interestingly, the model reproduces many of the clinical features observed in patients with germline mutations in *SBDS*, known as Shwachman-Diamond syndrome, and *Sbds* loss restricted to hematopoietic progenitors does not fully recapitulate the disease⁹⁶. Further analysis of these mice has uncovered a mechanism by which the mesenchymal niche creates a genotoxic environment capable of inducing DNA damage in HSPCs, thereby encouraging leukemogenesis⁹⁷. An analogous study inspired by patients with Noonan syndrome reported that conditional expression of mutant *Ptpn11*, a positive regulator of Ras signaling, in MSCs gives rise to a myeloproliferative disorder resembling juvenile myelomonocytic leukemia (JMML)⁹⁸. In contrast, hematopoietic cell restricted expression of the mutant protein produced a much milder phenotype. Similarly, osteoblastic expression of activated β -catenin induces acute myeloid leukemia in mice⁹⁹. The above observations demonstrate that at least in mouse models, where the niche cell population is uniformly mutated, a defective environment can ignite malignancy. Several other bone marrow-failure syndromes predispose patients to MDS or AML (e.g. Diamond-Blackfan anemia, dyskeratosis congenita, severe congenital neutropenia), begging the question of whether genetic changes in niche cells can initiate malignant transformation in humans.

3.3. Molecular alterations in human stromal cells: passenger or driver?

Mutations in hematopoietic cells give rise to clonal disorders, but whether inciting molecular lesions occur in bone marrow stromal cells remains a matter of debate. The primary conceptual hurdle, in the absence of a germline event, is how changes in one or a few niche cells can sufficiently influence malignant transformation given that the MSCs are largely quiescent and immobile in adult bone marrow³⁶. Nonetheless, extensive characterization of MSCs from patients with myeloid neoplasms unrelated to a heritable condition has revealed an array of molecular alterations including chromosomal abnormalities^{100, 101}, gene mutations¹⁰², and aberrant transcriptional and epigenetic signatures^{102, 103}, as well as functional changes in MSC secreted factors and differentiation potential^{97, 101, 104}. Some of these human data align closely with existing models such as increased activation of β -catenin in osteoblasts driving increased Notch signaling in leukemia initiating cells (LICs)⁹⁹. While intriguing and suggestive of biological plausibility, these correlative data cannot delineate the sequence of molecular events, and several convergent mechanisms of pathway activation may exist. Moreover, the lack of any recurrent genetic alterations in stromal cells across studies argues that some changes may be sporadic in the setting of an already

deregulated niche; however, selective sampling of stromal cells, which are relatively fixed compared to their fluid hematopoietic neighbors, could limit clinical detection of relevant mutations⁹³. Probably the most convincing evidence that niche cells can trigger disease comes from the stem cell transplant literature where cases of donor-derived leukemia have been reported¹⁰⁵. The caveat here is that the recipient niche has been altered by chemotherapy and/or radiation prior to transplant, and it is unclear whether the donor cells had preexisting mutations. Nevertheless, some extrinsic factor, iatrogenic or otherwise, skewed the healthy donor HSCs toward leukemogenesis.

3.4. Mutant hematopoietic cells reprogram their niches

Instead of being an initiator, the niche is more likely a facilitator of hematopoietic malignancies. Evidence for aberrant hematopoietic cells actively reprogramming their surrounding niche into an engine for disease propagation has been shown across a range of bone marrow diseases. Clinical and experimental findings have clearly established the reciprocal ability of malignant hematopoietic cells with discrete genetic alterations to remodel their surrounding niche. Live imaging of transplanted leukemia cells in mouse calvarium has visually captured active niche disruption¹⁰⁶, confirming what has long been suspected based on gross histologic changes in leukemic BM¹⁰⁷. Excitingly, in vivo and ex vivo models have allowed dissection of the cellular and molecular mechanisms that mediate these changes. Chronic myeloid leukemia (CML) cells elaborate a variety of cytokines (G-CSF, TNF α , IL-1 α , IL-6, TPO, CCL3, PIGF) that are associated with enhanced growth, irregular homing/retention of LICs, osteoblast expansion, and myelofibrosis¹⁰⁸⁻¹¹⁰. Multiple myeloma (MM) cells spur lytic bone disease via upregulation of receptor activator of NF- κ B ligand (RANKL) and other chemokines (CCL3, IL-3, IL-6, MMPs) that enhance osteoclast activity and impair osteoblast differentiation¹¹¹. Abnormal megakaryocytes in primary myelofibrosis (PMF) secrete proinflammatory paracrine signals (e.g. TGF- β , PDGF)¹¹² and two recent publications, one authored by our group, have demonstrated that aberrant MSC differentiation gives rise to excess myofibroblasts that cause BM fibrosis^{113, 114}. A parallel mechanism of IL-1 β -mediated damage of neuronal cells by JAK2V617F MPN cells has been reported as well¹¹⁵. Similarly, AML cells unleash pleomorphic effects on osteolineage cells^{116, 117}, adipocytes¹¹⁸, endothelial cells¹¹⁹, and sympathetic neurons¹²⁰, in turn promoting clonal expansion, skewing MSC differentiation, and downregulating factors required for normal HSPC function (e.g. CXCL12, SCF). Lastly, in a co-culture system, MDS hematopoietic cells induce transcriptional changes in mesenchymal cells that facilitate the engraftment and disease progression in a murine xenograft model¹²¹. Many of these modes of niche deregulation have been targeted in preclinical models and shown to modify disease progression, arguing that the niche plays a key evolutionary role in malignant transformation, even if it is not the primary driver.

3.5. Advanced hematopoietic malignancies may escape niche dependence

Many of the environmental changes induced by malignant hematopoietic cells are not simply reactive but help to establish clonal dominance at the expense of normal hematopoiesis. This has achieved twofold: 1) downregulation of critical factors required for normal HSPC maintenance; and 2) provision of positive feedback signals (e.g. cytokines, growth factors, cell-cell contact) from niche cells that confer a growth advantage to

malignant cells. The extent to which niche changes support disease propagation likely depends on spatial and temporal circumstances throughout the course of disease. Subclinical disease starts with premalignant hematopoietic cells that intuitively rely more on niche interactions for survival. Very few genetic alterations distinguish them from their normal counterparts, and they remain receptive to niche crosstalk. Progression to a low or intermediate grade neoplasm may start to disrupt normal hematopoiesis but does not permit niche independence, as demonstrated by the need to co-transplant human MSCs and MDS cells together in order to achieve efficient engraftment in immunodeficient mice¹²¹. Similarly, pre-leukemia cells rely on Wnt signaling and interactions with endosteal cells for proper homing/engraftment¹²². Upon conversion to a more aggressive disease state such as AML, intrinsic genetic and epigenetic alterations become the dominant molecular drivers and malignant HSPCs grow increasingly autonomous. This is supported by the fact that AML cells with the MLL-AF9 translocation are highly transplantable, no longer restricted to the HSC compartment, and generally less reliant on direction from the niche^{30, 122}. Similarly, T-ALL cells promiscuously interact with the bone marrow and do not depend on specific bone marrow niche factors for proliferation¹²³. Despite this gradual uncoupling from the niche, there are certain contexts where the microenvironment may continue to be important.

3.6. Targeting the niche to enhance eradication of malignant cells

Treatment failure and relapse are all too common realities in the field of malignant hematology in large part due to difficulty eliminating minimal residual disease (MRD). Specialized BM niches may confer drug resistance and/or maintain malignant cells in a dormant state that makes them impervious to standard chemotherapy. Disrupting these protective niche interactions has gained considerable interest as a therapeutic strategy. One approach has been to mobilize MRD cells by targeting the CXCL12-CXCR4 axis involved in HSC niche retention or by directly blocking adhesion molecules. Studies in xenograft models have provided early proof of concept that CXCR4 antagonists can mobilize leukemia cells and enhance disease eradication in combination with standard therapies^{124, 125}. Early phase clinical results have demonstrated feasibility and encouraging rates of remission in relapsed/refractory AML patients¹²⁶. Alternatively, differential adhesion molecule dependence between malignant and nonmalignant HSPCs has generated interest in selective blocking of various anchoring mechanisms that may be important in promoting quiescence and chemoresistance. Potential targets include CD44, VCAM-1/VLA-4, and E-selectin, for which drugs are already clinically available. The opposite approach to mobilization would be to somehow prevent MRD cells from reconstituting fulminant disease. Myeloma cells can remain dormant for years following treatment, and recent evidence suggests that close association with the endosteal niche maintains dormancy until osteoclast activity triggers relapse¹²⁷. Maintenance therapy directed at osteoclasts, for example with a bisphosphonate¹²⁸, would be a potential niche-directed intervention that could prolong remissions.

3.7. Premalignant States: a window of opportunity?

The old adage goes “prevention is better than cure,” speaking to the fact that thwarting onset of disease is the most effective way to preserve health. New molecular technologies, namely next generation sequencing, have enabled earlier detection of precancerous hematologic

lesions; however, the challenge is figuring what to do with this information, because the prognostic significance remains uncertain and there are currently no meaningful interventions.

A newly recognized premalignant state preceding MDS/AML called clonal hematopoiesis of indeterminate potential (CHIP) or age-related clonal hematopoiesis has emerged as a result of ubiquitous exome sequencing¹²⁹. Rates of transformation are estimated to be 0.5-1% per year¹³⁰. While acquisition of additional mutations appears to be requisite for transformation, chemotherapy has been shown to apply positive selective pressure¹³¹, and other niche factors may contribute as well. This formative stage may represent a window of opportunity to pursue aggressive preventative strategies to minimize the risk of transformation and help bolster innate mechanisms of abnormal clone clearance. Likewise, monoclonal gammopathy of undetermined significance (MGUS) denotes a benign accumulation of clonal plasma cells and a precursor state to MM, with a risk of transformation of about 1% annually¹³². Genome sequencing has demonstrated that nearly all the genetic alterations found in MM can also be found in MGUS, and small studies looking at serial samples before and after conversion have revealed a limited number of new mutations¹³³. Extrinsic factors from the microenvironment are therefore proposed to constrain the development of clinical malignancy and early evidence suggests a potential immune-mediated process¹³⁴. The precise nature of the MM niche has yet to be fully characterized, although it is plausible that IL7+ bone marrow stromal cells are involved as these cells are important for normal B cell development^{28, 29}. The key challenge moving forward will be identifying molecular mechanisms that render these cells dependent on the niche and designing relatively specific ways to intervene on them.

4. Conclusions and outlook

Our knowledge on the bone marrow niche has advanced considerably over the past 10 years. With more molecular and cellular players identified, novel mechanisms of maintaining HSCs and other hematopoietic cells are sure to emerge. These mechanisms will provide exciting new therapeutic paradigms for therapeutic intervention in hematologic diseases. Together, the available data support a model whereby cell intrinsic genetic and epigenetic changes are necessary but not always sufficient for full malignant transformation, and clinical disease depends on a permissive microenvironment. Outside of targeting BCR-ABL in CML, targeted cell intrinsic therapeutic strategies have proven insufficient in the management of clinical disease. Even in the case of CML, residual LICs have been proposed to rely on the niche to escape therapy¹³⁵. By defining and targeting more mechanisms of how malignant hematopoietic cells gain fitness in the niche, therapeutic intervention on niche mechanisms holds great promise.

Acknowledgments

This work is supported by the Rita Allen Foundation and the National Heart, Lung and Blood Institute (R01HL132074) and the Irma Hirschl Trust. S.S. was supported by the National Cancer Institute of the National Institutes of Health under Award Number T32CA203703. M.D. was supported by the Columbia Medical Scientist Training Program and NIH (1F30HL137323).

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Figure 1. Regulation Of Hematopoiesis By The Bone Marrow Microenvironment

a. Hematopoietic stem cells (HSC) are maintained and regulated by a variety of signals and cell-types in their perisinusoidal niche. Sinusoidal endothelial cells (Sinusoidal EC) and perivascular mesenchymal stromal cells (Perivascular MSC) support HSC self-renewal by release of SCF and CXCL-12. Mature hematopoietic cells and non-myelinating Schwann cells play roles in HSC quiescence and localization through various pathways, including TGF- β and CXCL4 signaling. A variety of physiologic and systemic cues modify HSC behavior through both direct and indirect signaling.

b. The bone marrow microenvironment regulates downstream hematopoiesis. Osteoblasts, MSCs, and mature hematopoietic cells support multipotent and committed progenitors, and are crucial players in efficient lympho-, myelo-, and erythropoiesis.

Figure 2. Schematic of proposed mechanisms by which the bone marrow microenvironment influences malignant transformation

- a. Premalignant hematopoietic cells are at a disadvantage during subclinical disease and constrained by highly evolved extrinsic signals from the native bone marrow microenvironment. These cells may be eliminated entirely or allowed to persist in a permissive state.
- b. A variety of local and systemic environmental factors, including stromal cell injury, can facilitate progression toward malignancy by disrupting homeostatic inhibitory signals and promoting secondary genetic events in premalignant hematopoietic cells.
- c. Malignant hematopoietic cells remodel the microenvironment. 1. Endosteal cells are hijacked by malignant plasma cells in multiple myeloma. Osteoclasts engage in abnormal bone resorption and osteoblasts provide a haven for malignant clone maintenance. 2. Deranged mesenchymal cells support malignant cell growth at the expense of normal hematopoiesis in MDS and AML. 3. Mesenchymal cells are directed toward myofibroblast differentiation in myelofibrosis. Extracellular matrix changes, including collagen and reticulin deposition, create a pro-inflammatory, fibrotic milieu that disrupts normal hematopoiesis.