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The bone marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites

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Abstract

In post-fetal life, hematopoiesis occurs in unique microenvironments or 'niches' in the marrow. Niches facilitate the maintenance of hematopoietic stem cells (HSCs) as unipotent, while supporting lineage commitment of the expanding blood populations. As the physical locale that regulates HSC function, the niche function is vitally important to the survival of the organism. This places considerable selective pressure on HSCs, as only those that are able to engage the niche in the appropriate context are likely to be maintained as stem cells. Since niches are central regulators of stem cell function, it is not surprising that molecular parasites like neoplasms are likely to seek out opportunities to harvest resources from the niche environment. As such, the niche may unwittingly participate in tumorigenesis as a leukemic or neoplastic niche. The niche may also promote metastasis or chemo-resistance of hematogenous neoplasms or solid tumors. This review focuses on what is known about the physical structures of the niche, how the niche participates in hematopoiesis and neoplastic growth and what molecules are involved. Further understanding of the interactions between stem cells and the niche may be useful for developing therapeutic strategies.

Keywords

niche; hematopoietic stem cells; osteoblasts; endothelial cells; mesenchymal stem cells; metastasis

Introduction

For species to survive and reproduce to maintain their populations, they must harvest resources from and be in tune with the environment that they find themselves in. Many combinations of environmental factors are necessary to prosper in the physical environment, to obtain energy and nutrients, to avoid predation and to ultimately pass on genetic material

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to progeny. These requirements are abstractly termed the 'ecological niche'. Historically, Grinnell proposed the 'niche' term as a collection of environment conditions where a species lives.[1] Hutchinson made the ecological niche concept popular by suggesting that the niche could be modeled as an imaginary space with many dimensions.[2] In mammals, Schofield proposed the niche hypothesis to describe the microenvironment that supports hematopoietic stem cells (HSCs).[3]

In the marrow, bone marrow stromal cells derived from mesenchymal stem cells (MSCs) are believed to provide the basis for the physical structures of the niche. As such, bone marrow stromal cells are thought to regulate self-renewal, proliferation and differentiation of the HSCs through production of cytokines and intracellular signals that are initiated by cell-to-cell adhesive interaction.[4] Bone marrow stromal cells are composed of cells of mesenchymal origin known to include osteoblasts, endothelial cells, fibroblasts and adipocytes.[5] As early as the 1970s, studies examining the interaction between bone marrow stromal cells and HSCs had already suggested a unique and critical role for events localized at endosteal surfaces, and by inference osteoblasts, as having a central role in hematopoiesis.[6,7] Only recently have studies demonstrated that osteoblasts comprise a crucial component of the HSC niche ('endosteal niche'),[8–11] while cells of other lineages including endothelial cells (for example, 'vascular niche') are also likely to participate in niche functions.[12–15] At the same time, there is a growing appreciation from studies examining the physical locale of where MSCs reside in marrow that they too may localize to similar niches in marrow[16] (Figure 1a). As such, it is very likely that HSCs and MSCs are intimately associated with one another. Moreover, it is likely that the co-coordinated activities of the niche (be it endosteal or endothelial) and all the stem cells collectively are responsible for integrating the delicate balance between hematopoiesis and tissue turnover.

Many solid tissue neoplasms also have a predisposition to metastasize to the marrow, including breast and prostate cancers (PCa).[17] Multiple factors are involved in the metastatic spread of neoplasms to the marrow. Potentially these tumor cells have the ability to respond to niche localization signals that promote extravasation from primary sites as well as the preferential adhesion to bone marrow endothelium. In addition, the niche may add to or participate in the selection of malignant clones that facilitate the adaptation of cancer cells to grow within bone marrow microenvironments.[17,18] On the basis of our studies relating to the HSCs homing, we have recently reported the significant role of the chemokine CXC chemokine ligand 12 (CXCL12 or SDF-1)/its major receptor CXC chemokine receptor 4 (CXCR4) axis on the bone metastasis of Pca.[19–23] Although the mechanisms of bone metastasis are still unclear, the CXCL12/CXCR4 chemokine axis is likely to play a significant role in regulating the bone metastasis of solid neoplasms. Thus, several lines of evidence demonstrate that bone marrow stromal cells are also able to promote the growth, survival and drug resistance of hematogenous neoplasms.[24–26] These findings suggest that the molecular mechanisms used by the niche to maintain quiescence of stem cells may be parasitized to facilitate neoplastic growth.[27]

In this review, we focus on the role of bone marrow stromal cells, with a particular focus on osteoblasts, in the HSC niche. We also explore and develop the hypothesis that neoplasms function as molecular parasites on the niche (Figure 1b). By taking advantage of the normal

machinery used by the niche to maintain stem cells as stem cells, we believe that tumors are able to survive in the marrow for long periods of time. Therefore, the development of niche-directed therapies would add to the armamentarium of therapies for leukemia and bone metastasis of solid neoplasms.

Hematopoietic stem cells niche

In adults, marrow-derived HSCs are the principle cells of origin of all mature hematopoietic cell phenotypes. Hematopoiesis is considered as a pyramidal/hierarchical process with the cells' of greatest maturation potential or primitiveness sitting at the top of the hierarchy, and cells that have undergone terminal differentiation at the bottom. Terminally differentiated blood cells are generally classified into one of two major lineages: those derived from myeloid lineages and those derived from lymphoid progenitors. Myeloid cells include red blood cells, platelets and cells responsible for cellular immunity, such as macrophages and granulocytes. Cells derived from lymphoid progenitors are major participants in coordinating humeral and cellular immunity.

Experimental data suggest that HSCs differentiate into hematopoietic progenitor cells that are capable of exponential proliferation as well as continuing the process of differentiation. Alternatively, HSCs are capable of self-replicate activity, which may give rise to one or two identical daughter cells. As a result, HSC activity must be tightly regulated so as to meet physiologic demands, but also to protect HSCs from oncogenic, physical and chemical damage. The site or physical locale that regulates self-renewal, proliferation and differentiation of HSCs has been termed the 'HSC niche' (Figure 2). The niche is likely comprised of many different niche constituents including osteoblasts, endothelial cells, fibroblasts and adipocytes. Other cells known to participate in the generation of the niche or its regulation are osteoclasts and the HSCs and progenitor cells themselves—which is only now becoming appreciated.[28]

Osteoblasts

As late as 1992, virtually nothing was known regarding the role of osteoblasts in hematopoiesis. What was published was focused on what cytokines/growth factors were produced by osteoblast, the role that osteoblasts play in regulating osteoclastic activities or on the role that immortal cell lines play in hematopoiesis.[29] Since this time, our group has demonstrated that osteoblasts support hematopoietic progenitor and long-term culture-initiating cell (LTC-IC) activities in a human system.[30–32] Moreover, the proximity of CD34-positive bone marrow cells to osteoblasts can induce the synthesis of several cytokines, including interleukin (IL)-6,[33] leukemia inhibitory factor (LIF),[33] transforming growth factor-β1 (TGF-β1),[31] macrophage inhibitory protein-1α,[34] hepatocyte growth factor (HGF),[32] CXCL12,[35] and IL-7.[35] However, progenitor cells and LTC-IC survival on osteoblasts requires direct cell-to-cell contact, despite the elaboration of soluble cytokines that are necessary, but clearly not sufficient for maintenance of hematopoietic cells on osteoblasts.[30] HSCs also bind tightly to osteoblasts *in vitro*, and that these interactions were sensitive to trypsin, chelators and glycosylation inhibitors.[36] These data suggest that direct cell-to-cell contact of HSCs on osteoblasts is critical to ensure HSCs survival (Figure 2).

Although osteoblasts express many cell adhesion molecules that may be used during HSCs–osteoblasts adhesion, the molecular mechanisms resulting the interactions between HSCs and osteoblasts are not well defined.[29] In recent work, we have demonstrated that the adhesion between HSCs and osteoblasts is mediated by cell-to-cell rather than cell-to-matrix.[5,36] Moreover, two separate types of adhesive relationships seem to exist.

One group of cell adhesion molecules regulates the immediate binding of HSCs homing to the endosteal niche. Rapidly processed N-linked glycosylated proteins constitutively expressed on HSCs, as detected using selective pretreatment with glycosylation inhibitors, are most likely involved.[36] CXCL12/CXCR4 interactions are also thought to regulate localization of HSCs to endosteal niche,[37,38] and play a major role in granulocyte colony-stimulating factor (G-CSF) induced HSCs mobilization.[39] CXCL12 is a critical component of the niche that appears to be a pivotal point of regulation of cell entry and egress from the marrow. The interactions between CXCL12 and its major receptor CXCR4 are thought to play an important role in normal hematopoietic development.[17,40] Animal studies have shown significant defects in the marrow function in the absence of CXCL12 or CXCR4.[41–44] In addition, blockade of CXCR4 prevents HSC homing to the marrow.[45] CXCL12 is constitutively produced by many bone marrow stromal cells including endothelial cells and fibroblasts, although osteoblasts are likely to be the major a source of the protein under basal conditions.[5,37,38] Yet, other cells may in fact be capable of expressing CXCL12 and presenting it to HSCs including the recently described CXCL12-abundant reticular cells.[46] Our recent data also demonstrate that annexin II plays an important role in the localization of HSCs to the endosteal niche[47] (Figure 2). Such that blocking annexin II with antibody or competing peptide prevents engraftment and survival in lethally irradiated animals.[47]

Another group of adhesion molecules probably provides survival signals or generates activation of a quiescent state in HSCs after they are localized to the endosteal niche. These are known to include ostepontin (OPN),[48,49] very late antigen-4 (VLA-4)/fibronectin (FN) or vascular cell adhesion molecule-1 (VCAM-1),[50] leukocyte function associated antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1),[50] and N-cadherin.[9,11] (Figure 2)

Although HSCs and osteoblasts are closely associated with each other in the bone marrow, and osteoblasts modulate normal hematopoiesis,[5] the details of the post-receptor engagement events that regulates HSC quiescence remains largely unexplored. One pathway that has garnered considerable attention is the N-cadherin and β-catenin pathway, are believed to be in part responsible for interaction between osteoblasts and long-term HSCs,[9] although recent reports suggest that HSCs themselves may not express N-cadherin.[51] Likewise, activation of osteoblasts through the parathyroid hormone receptor (PTHr) has been shown to expand HSCs number.[10] Here, osteoblasts are thought to regulate HSCs number through Notch1/Jagged1 pathway signaling.[10] Likewise, the tight adhesion between HSCs and osteoblasts induces Tie2/angiopietin 1(Ang-1) signaling is likely to result in the maintenance of HSCs in a quiescent state in the bone marrow niche.[11] Along these lines, most recently we demonstrated that osteoblasts directly support B lymphopoiesis through VLA-4/VCAM-1 interaction[35] as a function of osteoblasts to regulate the differentiation of

HSCs. These findings strongly suggest a relationship between osteoblasts and hematopoietic cells, but the dimension of this interaction has, yet, to be completely defined.

Endothelial cells

Recent work by several groups suggest that not only do bone marrow endothelial serve to partition the vascular and extra-vascular marrow spaces, but they also regulate HSCs by functioning in a niche capacity. The vast majority of the studies support that an endothelial marrow niche does so on account of the localization of HSCs by immunohistochemical analysis. The most eloquent being the use of simple combinations of markers, which are termed 'SLAM' family receptors, including CD150, CD244 and CD48, which identified the majority of HSCs in close physical association with endothelial cells *in vivo*.[12] The presence of a bone marrow endothelial cell, or 'vascular niches' for HSCs makes inherent sense in that in order to rapidly mobilize large quantities of stem cells into the periphery they would need to be in position and ready to respond to systemic challenges. However, a substantial body of literature has demonstrated that transplanted HSCs localize rapidly to the endosteum,[48,52] and marrow dissection studies provide similar information.[53,54] Yet, whether there is a circulation or communication between the two niches (vascular and endosteal), has been difficult to identify directly.[15]

What has been dissected from the system is that CXCR4 expressed on the bone marrow endothelial cells plays an important role in the homing of human CD34-positive hematopoietic progenitors to the bone marrow by translocation of circulating CXCL12 into the bone marrow.[13] Bone marrow endothelial cells also induce HSCs proliferation and expansion by secreting the growth factors such as insulin-like growth factor binding protein-3 (IGFBP-3).[14] Thus, endothelial cells might regulate HSC function and could contribute to the creation of HSC niches as they do in the nervous system.[12]

Like their role in supporting HSCs, bone marrow endothelial cells play a critical role in thrombopoiesis.[55] CXCL12 and fibroblast growth factor-4 (FGF-4) induced the localization and adhesion of megakaryocytes to the bone marrow endothelial cells.[55] The direct contact between megakaryocyte and bone marrow endothelial cells promotes survival, maturation and release of platelets through VE-cadherin and/or VLA-4/VCAM-1 axis.[55] Similarly, the VLA-4/VCAM-1 axis is likely to play a critical role in HSC trafficking.

Although it is still controversial, several reports suggest that HSCs in different niches are in different states of quiescence. One attractive model for HSCs maintenance and expansion is that osteoblasts mainly maintain the quiescent state of HSCs, while HSCs differentiate and proliferate on endothelial cells[56] (Figure 2). If true, HSCs in an endosteal niche may be more deeply quiescent than in vascular niches. Such that in response to stress as in infection, allergy or bleeding, HSCs are able to enter into replicative states in an vascular niche or migrate to an vascular niche, and are capable of entering the circulation. This process, in reverse would provide a mechanism for induction of quiescence in endosteal sites or niches. Of course this hypothesis is highly speculative and an area of intense investigation and debate.

Mesenchymal stem cells

MSCs have the potential to differentiate into specialized cells of mesenchymal origin, including adipocytes, chondrocytes, myoblasts and osteoblasts.[57–63] However, MSCs are present at low frequency in bone marrow and in the circulation.[64] Despite the lack of cellular and molecular tools and *in vivo* assays that permit the rigorous identification of stem cells from mesenchymal tissues, it is known that a cell population with MSC-like characteristics can be isolated based on its ability to adhere to plastic and may be partially purified by separation techniques.[65–68] MSCs can be expanded to great numbers by *in vitro* culture, however, such a step is time consuming, expensive and risks cell contamination. Most importantly, as these cells gain or lose their differentiation potential during long-term culture, they likely acquire properties that do not appear under physiologic, *in vivo* circumstances. Therefore, studies have demonstrated that pure MSCs are quite rare. Furthermore, limiting our efforts to *in vitro* studies aimed at expanding MSCs without adequate *in vivo* assays hinder the rigorous identification of stem cells from mesenchymal tissues. In parallel, our *in vivo* work by referring to other work[69] has demonstrated that a 5-fluorouracil (5-FU) resistant population of mesenchymal cells is capable of multi-lineage differentiation. Likewise, it has recently been demonstrated that a CD146-positive cells in human marrow is able to perform a similar function *in vivo*.[70]

It is known that the endosteal/subendosteal microenvironment includes bone lining and adjacent reticular cells that support HSC self-renewal, proliferation and differentiation. Recently, a technique was developed to fractionate the marrow using differential digestion.[16] The two distinct stromal-cell populations containing the fraction of subendosteal reticulocytes and osteoblasts were isolated from periosteum-free fragments of murine femurs by a two-step collagenase-digestion procedure. Both populations produce similar extracellular matrix (collagen I, laminin, FN and decorin), except for collagen IV, which is low in a fraction of subendosteal osteoblasts. These populations are distinctly different in their osteoblastic characteristics *in vitro* compared to marrow that is flushed (whole bone marrow). In conjunction with preconditioning mice with 5-FU, we subsequently demonstrated that the fraction of subendosteal reticulocytes population had a greater osteogenic potential than the other marrow fractions *in vitro* and *in vivo* animal model[71] (in preparation).

For identification of HSCs, the answer has been to eliminate the tissue through lethal levels of radiation. For MSCs or their immediate progeny, this approach is not feasible due to the lack of an assay comparable to the competitive reconstitution assay. However, recent work described a method of multiparameter cell sorting that yielded a population of cells that resembled very primitive, in fact, embryonic-like cells when cultured.[72] These cells develop into neurospheres and their potential for tissue/organ regeneration appears to be unlimited.[72] It has also been identified as a population of CXCR4-positive/lineage-negative/CD45-negative cells that express SSEA, Oct-4 and Nanog in adult bone marrow.[73] These cells are extremely small and display several features typical for primary embryonic stem cells. These cells include (1) a large nuclei surrounded by a narrow rim of cytoplasm; (2) open-type chromatin (euchromatin) and (3) high telomerase activity.[73] In *in vitro* cultures, these cells are able to differentiate into all three germ-layer lineages. The number of these cells is

highest in the bone marrow from young (approximately 1-month old) mice and decreases with age. It is also observed that significantly fewer cells are found in the short-living DBA/2J mice as compared to long-living B6 animals. These cells strongly respond *in vitro* to CXCL12, HGF/scatter factor and LIF.[72] They also express CXCR4, c-met and LIF receptor.[72] The authors named these cells very small embryonic-like (VSEL) stem cells, and hypothesized that they are direct descendants of the germ lineage. These combinations of markers go along with the concept that these cells may in fact be embryonic stem cells, epiblast stem cells and primordial germ cells.[72–77] These cells could also give rise to MSCs down the road. Thus, VSEL cells could reside on the top of the MSC hierarchy in bone marrow. Moreover, the presence of these stem cells in adult tissues including bone marrow, epidermis, bronchial epithelium, myocardium, pancreas and testes supports the concept that adult tissues contain some population of pluripotent stem cells that are deposited in embryogenesis during early gastrulation. In fact, the cells deposited in the marrow early during ontogenesis and can be mobilized from bone marrow and circulate in peripheral blood during tissue/organ injury in an attempt to regenerate damaged organs.

Further studies have demonstrated that VESL cells, when isolated freshly from bone marrow, neither grow hematopoietic colonies nor radioprotect lethally irradiated recipients. However, when VSEL cells are co-cultured with C2C12 murine sarcoma-supportive feeder layers, the sphere formal cells, which are composed of immature cells with large nuclei containing euchromatin and express other VSEL cell-like properties, can be subcultured[78] After replating these cells, new embryonic-like bodies were formed. If plated into cultures promoting tissue differentiation, VSEL cells show pluripotency and expand into cells from all three germ-cell layers. In this case, however, CD45-negative VSEL, if cultured/passaged in methylocellulose cultures supplemented with hematopoietic growth factors (c-kit ligand, IL-3, erythropoietin (Epo) and granulocyte-macrophage colony-stimulating factor (GM-CSF)), give rise to colonies composed of myeloid ($CD45^{-}$positive/Gr-1-positive) and erythroid (Ter119-positive) hematopoietic cells. The hematopoietic differentiation of VSEL cells was accompanied by upregulation of mRNA for several genes regulating hematopoiesis (for example, PU-1, c-myb, LMO2 and Ikaros).

From the standpoint of the niche, VSEL cells are highly mobile and respond to a CXCL12 gradient and adhere to bone marrow-derived fibroblasts. Time-lapse studies reveal that the cells attach to and migrate beneath and/or undergo emperipolesis in bone marrow stromal cells. Interaction of VSEL cells with bone marrow stromal cells is inhibited after preincubation of the cells with CXCR4 antagonists. Since bone marrow stromal cells secrete CXCL12 and other chemoattractants, stromal cells may create a homing environment for VSEL cells. Thus, the interaction of VSEL cells with MSCs that are isolated from bone marrow stromal cells may, in fact, have been ‘contaminated’ by VSEL cells including the so-called multipotential adult progenitor cells, unrestricted somatic stem cells or marrow-isolated adult multilineage inducible cell cultures.[79] On the basis of these observations and observations that bone marrow stromal cells support HSC growth and differentiation, it is clear that ‘MSCs’ reside in bone marrow in a locale that is similar to HSCs (Figure 1a).

In a parallel line of work, it has been demonstrated that co-transplantation of enriched MSCs with HSCs improves engraftment and reduces graft failure (or graft-versus-host disease).

[80–82] Likewise, co-culture of HSCs with mixed MSCs facilitates the expansion of HSCs ex vivo to a limited degree.[83–85] Consequently, MSCs are likely to be an essential component of HSC niche, as well as osteoblasts and endothelial cells. Part of the mechanism may be that MSCs provide support during hematopoiesis through the abundant production of hematopoietic-supportive molecules such as fibronectin, osteopontin, CXCL12, Ang-1, thrombospondin and others.[86] MSCs are also rich sources of other hematopoietic active cytokines including but not limited to IL-6, −7, −8, −11, −12, −14, −15, LIF, macrophage (M)-CSF, fms-like tyrosine kinase-3-ligand (Flt3-L) and stem cell factor.[87] Yet, the production of these molecules by MSCs has not been clearly identified.

Besides expanding HSCs, MSCs can support T-cell [86,88,89] and B-cell[88,90] survival in a quiescent condition by preventing apoptosis. In addition, MSCs inhibits dendritic cell differentiation[91,92] by inducing cell cycle arrest. By inference with the activities of MSCs then, the main roles of MSCs in hematopoiesis are thought to regulate the immune system. Although the role of the niche as an entity that regulates immunity is relatively unexplored, the concept that MSCs may regulate engraftment and event metastasis is clearly an area in need of further investigation.[93]

Molecular parasites of the niche

It was recently proposed that most cancers contain a rare population of functionally distinct cancer stem cell (CSC). One of the first neoplasms in which a stem cell was identified was acute myeloid leukemia (AML).[94] In this disease, the frequency of the leukemic stem cell (LSC) is approximately one per million AML blasts where a CD34-positive/-negative cell fraction representing 0.1–1% of the neoplasm cells possessed all the leukemia-initiating activity in the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) transplantation model.[94] A mammary CSC has recently been isolated from primary mammary carcinomas using four cell-surface markers (CD44, CD24, a mammary cancer marker and epithelial-specific antigen).[95] The neoplasm-initiating capacity of the cells was also verified in an *in vivo* NOD/SCID engraftment assay. The mammary CSCs represented approximately 2% of the unfractionated bulk neoplasm cells.

As was described previously, stem cells generally reside in a microenvironmental niche, where niche regulates proliferation, differentiation and self-renewal of the stem cells. However, an open question is where in marrow the CSC niche is and how it does function. An equally important question to discern whether or not, the ‘normal’ niche is one and the same with those targeted by metastatic cells. If so, does the function of the niche itself contribute to metastasis? Intuition would suggest engagement of the niche by similar adhesive mechanism used by HSC to localize to the niche would facilitate neoplastic dissemination.

We have approached the issue from the perspective of PCa, because PCa is a common cancer and the second leading cause of cancer deaths in American men.[19,20,96,97] The high-mortality rate is principally attributable to the spread of malignant cells to many tissues including bone.[19] Although the most common metastatic site of PCa is the bone, there have not been many advances in the therapeutic arena to prevent or diminish these lesions. Bone

provides chemotactic factors, adhesion factors and growth factors that allow the PCa to target and proliferate in the bone.[96] CXCL12 is thought to activate proliferation of PCa cells.[20,21] On the other hand, neoplasm-derived factors have direct effects on osteoblasts differentiation and proliferation, the response of bone to neoplastic invasion and bone homeostasis. In a reciprocal relationship, PCa is believed to supply osteoblastic growth factors, such as vascular endothelial growth factor (VEGF)[98] and osteolytic factors that modulate bone remodeling.[96] At the same time, while osteoblastic activity is clearly the predominant feature of late stage of PCa metastasis, there is a significant component of osteoclastic activity in PCa. PCa-induced osteolysis may be due to both inhibitory effects on osteoblasts and stimulatory effects on osteoclasts by enhanced OPN secretion by osteoblasts.[99] We have also reported that the CXCL12/CXCR4 chemokine axis plays a significant role in the bone metastasis of PCa,[19–23] by activating αvβ3 integrins[23] and CD164.[22] CD164 also may play an important role in localizing tumors not only to sites where there are high levels of CXCL12 expression, but also to specific tissue locales.[22] CXCR4 expression is correlated with neoplastic grade,[20] and that CXCL12 signaling through CXCR4 triggers the adhesion of PCa cells to bone marrow endothelial cells.[19] Recently we also found that the CXCL12/CXCR4 chemokine axis plays a significant role in secreting proangiogenic signals, such as IL-6, −8, tissue inhibitors of metalloproteinase (TIMP)-2 and VEGF, through MEK/extracellular signal-regulated kinase (ERK) or PI-3K/AKT pathway and in regulating expression of angiostain levels by using short interfering RNA knockdown technology for CXCR4.[97] Moreover osteoblasts are known to regulate PCa growth and survival. As such, PCa and osteoblasts regulate activities of each other, much like the relationship between HSCs and the endosteal niche.

An other open question is what does engagement of the niche mean specifically for tumors? One would expect that activation of the programs that are used by the niche to regulate quiescence in HSCs and possibly MSCs would result in the induction of dormancy programs in neoplasms. LSCs share many of the properties of HSCs including aspects pertaining to self-renewal, differentiation and lineage commitment pathways. As was detailed earlier, several members of the bone marrow stromal-cell family contribute either physically or functionally to the HSC niche. Likewise, bone marrow stromal cells control the fate of LSCs, including quiescence, resistance to apoptotic stimuli and resistance to drugs. These pathways are regulated most efficiently when LSCs are in near proximity to bone marrow stromal cells or in direct contact.[100] For example, direct cell-to-cell contact inhibits leukemic cells from cell growth and activates cell survival signaling pathways leading to the protection of LSCs from chemotherapy-induced apoptotic cell death.[101]

Although the molecular mechanisms which regulate LSC self-renewal remain unclear, a recent study demonstrated that asparagine, which is secreted by bone marrow stromal cells in large amounts, protected acute lymphoblastic leukemia (ALL) cells from asparaginase treatment in co-culture systems.[102] Some adhesion molecules such as VCAM-1, FN, hyaluronic acid and VE-cadherin were demonstrated to have prosurvival activity in leukemia.[24,103–106] Along these lines, direct cell-to-cell contact between leukemia and the niche may not only contribute to cell adhesion, but mediate resistance to chemotheraputic agents. For example, adhesion of B-lineage ALL cells to bone marrow stromal cells provides protection from cytarabine- and etposide-induced apoptosis through the VLA-4/VCAM-1

interactions.[101] VLA-4/FN interactions have also been shown to regulate drug resistance in AML by altering the PI-3K/AKT signaling pathways to compensate for interruption of VLA-4 signaling in the presence of function-blocking antibodies.[107] Likewise, CD44 is thought to play a key function in AML such that antibody to the molecule inhibits AML homing to the marrow.[108] Growth arrest following adhesive contacts between leukemic cells and the stromal niche appears to be critical juncture in protecting leukemic cells from drugs that induce apoptosis. Although microenvironment for leukemia is still unclear, a recent study has demonstrated that osteoblasts are capable of serving in a niche capacity for leukemic cells.[27] The bone marrow is thought to be not only primary site of leukemia, but also one of the sites for leukemic relapse. CXCL12/CXCR4 interactions provide ALL cells a pathway for homing to endothelial cells in the bone marrow, and subsequently an opportunity to engage the niche.[109] Conversely, leukemic cells actively recruit bone marrow endothelium to establish vascular networks suitable for continued growth via a CXCL12/CXCR4 pathway.[40,110] What are the molecules involved, and how osteoblasts regulate the leukemic niche is far from understood. As mentioned in the context of HSCs, osteoblasts produce a vast number of cell-associated and -secreted proteins that may induce growth arrest of leukemic cells.[5] Yet, none are likely to be sufficient alone. Clearly, a better understanding of the molecular mechanisms regulating the interactions between the niche and its constituents and leukemic cells will lead to new possibilities for therapeutic interventions in patients with minimal residual disease.

In view of these observations, it seems reasonable to surmise that many of the molecular mechanisms that enable self-renewal of HSCs may be shared with LSCs and CSCs (Figure 1b). One possibility is that the number of niches is limited, and therefore as tumor burden increases, the capacity for regulation is eventually overwhelmed. Under such a scenario, it is appealing to speculate that establishment of niche regulation, or expansion of the niches may be attractive therapeutic targets.

Summary

The bone marrow stromal cells provide survival signals to HSCs, LSCs and CSCs probably through similar pathways. Yet, although a number of similarities exist between normal HSCs, LSCs and CSCs in terms of molecular mechanisms that regulate self-renewal and interactions with the bone marrow microenvironment, clearly differences exist as well[111,112] (Table 1). One such mechanism that was recently proposed is differences in microRNAs, small nonprotein-coding RNAs, believed to regulate the self-renewal of stem cells. The loss of microRNAs in aging of stem cells may promote tumorigenesis.[113] The role of bone marrow stromal cells in protecting leukemic cells from chemotherapy through cell-to-cell adhesion has been widely recognized. One possibility is that LSCs have much more active migration machinery when compared with HSCs that allow them to escape growth inhibition or quiescence promoting signals induced by osteoblasts and other stromal cells in the niche. Thus, drugs that target adhesion molecules combining with current chemotherapy hold certain promise as the next step of therapeutic regimens to leukemia. Similar to hematopoiesis and leukemia, bone marrow stromal cells might involve in creating CSCs through direct cell-to-cell contact as a metastatic site. Although the present chemotherapies destroy the bulk of a tumor, they do not target specifically CSCs that may be slowly cycling

especially if they are engaged in the niche (Figure 3). Therefore, a next step of cancer chemotherapy will most likely be to target CSCs. On the other hand, CXCL12/CXCR4 chemokine axis may play an important role in localizing neoplasms to the niche. Thus, therapies that block the interaction between chemokine and chemokine receptors might prove to be efficacious therapeutic agents to prevent metastasis, as well.

In this review, we have focused on the important role of bone marrow stromal cells in HSC, LSC and CSC niches. As described above, the roles of MSCs as HSC niche participants have also recently been realized. As such, HSCs and bone marrow stromal cells, including osteoblasts, endothelial cells and MSCs, are believed to localize closely in the bone marrow (Figure 1a). They are also thought to affect each other and the function of the niche. Therefore, osteoblasts, endothelial cells and MSCs are also likely to be involved in the LSC or CSC niche, albeit unwittingly (Figure 1b).

Further understanding of the interactions between HSCs and bone marrow stromal cells, including osteoblasts, endothelial cells and MSCs, might be useful for new therapeutic strategies to the bone marrow transplantation. Targeting drugs to supporting cells of the niche or adhesion and other molecules might also amplify the efficacy of traditional chemotherapy for leukemia and cancers.

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References

1. Grinnell J. The niche relationships of the California thrasher. Auk. 1917; 34:427–433.
2. Hutchinson GE. Concluding remarks. Cold Spring Harb Symp Quant Biol. 1957; 22:415–427.
3. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978; 4:7–25. [PubMed: 747780]
4. Miura Y, Gao Z, Miura M, Seo BM, Sonoyama W, Chen W, et al. Mesenchymal stem cell-organized bone marrow elements: an alternative hematopoietic progenitor resource. Stem Cells. 2006; 24:2428–2436. [PubMed: 17071859]
5. Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood. 2005; 105:2631–2639. [PubMed: 15585658]
6. Patt HM, Maloney MA. Bone formation and resorption as a requirement for marrow development. Proc Soc Exp Biol Med. 1972; 140:205–207. [PubMed: 4555913]
7. Lord BI, Hendry JH. The distribution of haemopoietic colony-forming units in the mouse femur, and its modification by X-rays. Br J Radiol. 1972; 45:110–115. [PubMed: 4550739]
8. Taichman RS, Emerson SG. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J Exp Med. 1994; 179:1677–1682. [PubMed: 7513014]
9. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003; 425:836–841. [PubMed: 14574412]

10. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003; 425:841–846. [PubMed: 14574413]
11. Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 2004; 118:149–161. [PubMed: 15260986]
12. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005; 121:1109–1121. [PubMed: 15989959]
13. Dar A, Goichberg P, Shinder V, Kalinkovich A, Kollet O, Netzer N, et al. Chemokine receptor CXCR4-dependent internalization and resecretion of functional chemokine SDF-1 by bone marrow endothelial and stromal cells. Nat Immunol. 2005; 6:1038–1046. [PubMed: 16170318]
14. Chute JP, Muramoto GG, Dressman HK, Wolfe G, Chao NJ, Lin S. Molecular profile and partial functional analysis of novel endothelial cell-derived growth factors that regulate hematopoiesis. Stem Cells. 2006; 24:1315–1327. [PubMed: 16373696]
15. Petit I, Jin D, Rafii S. The SDF-1-CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol. 2007; 28:299–307. [PubMed: 17560169]
16. Balduino A, Hurtado SP, Frazao P, Takiya CM, Alves LM, Nasciutti LE, et al. Bone marrow subendosteal microenvironment harbours functionally distinct haemosupportive stromal cell populations. Cell Tissue Res. 2005; 319:255–266. [PubMed: 15578225]
17. Neiva K, Sun YX, Taichman RS. The role of osteoblasts in regulating hematopoietic stem cell activity and tumor metastasis. Braz J Med Biol Res. 2005; 38:1449–1454.
18. Cooper CR, Chay CH, Gendernalik JD, Lee HL, Bhatia J, Taichman RS, et al. Stromal factors involved in prostate carcinoma metastasis to bone. Cancer. 2003; 97(3 Suppl):739–747. [PubMed: 12548571]
19. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res. 2002; 62:1832–1837. [PubMed: 11912162]
20. Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, et al. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) *in vivo*. J Cell Biochem. 2003; 89:462–473. [PubMed: 12761880]
21. Sun YX, Schneider A, Jung Y, Wang J, Dai J, Cook K, et al. Skeletal localization and neutralization of the SDF-1(CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites *in vivo*. J Bone Miner Res. 2005; 20:318–329. [PubMed: 15647826]
22. Havens AM, Jung Y, Sun YX, Wang J, Shah RB, Buhring HJ, et al. The role of sialomucin CD164 (MGC-24v or endolyn) in prostate cancer metastasis. BMC Cancer. 2006; 6:195. [PubMed: 16859559]
23. Sun YX, Fang M, Wang J, Cooper CR, Pienta KJ, Taichman RS. Expression and activation of alpha(v)beta(3) integrins by SDF-1/CXC12 increases the aggressiveness of prostate cancer cells. Prostate. 2007; 67:61–73. [PubMed: 17034033]
24. Bewick MA, Lafrenie RM. Adhesion dependent signalling in the tumour microenvironment: the future of drug targetting. Curr Pharm Des. 2006; 12:2833–2848. [PubMed: 16918414]
25. Li ZW, Dalton WS. Tumor microenvironment and drug resistance in hematologic malignancies. Blood Rev. 2006; 20:333–342. [PubMed: 16920238]
26. Ramasamy R, Lam EW, Soeiro I, Tisato V, Bonnet D, Dazzi F. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on *in vivo* tumor growth. Leukemia. 2007; 21:304–310. [PubMed: 17170725]
27. Ninomiya M, Abe A, Katsumi A, Xu J, Ito M, Arai F, et al. Homing, proliferation and survival sites of human leukemia cells *in vivo* in immunodeficient mice. Leukemia. 2007; 21:136–142. [PubMed: 17039228]
28. Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K, Sztainberg Y, et al. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med. 2006; 12:657–664. [PubMed: 16715089]
29. Taichman RS, Emerson SG. The role of osteoblasts in the hematopoietic microenvironment. Stem Cells. 1998; 16:7–15. [PubMed: 9474743]

30. Taichman RS, Reilly MJ, Emerson SG. Human osteoblasts support human hematopoietic progenitor cells *in vitro* bone marrow cultures. Blood. 1996; 87:518–524. [PubMed: 8555473]
31. Taichman RS, Reilly MJ, Emerson SG. Human osteosarcomas inhibit hematopoietic colony formation: partial reversal by antibody to transforming growth factor-beta 1. Bone. 1997; 21:353–361. [PubMed: 9315339]
32. Taichman R, Reilly M, Verma R, Ehrenman K, Emerson S. Hepatocyte growth factor is secreted by osteoblasts and cooperatively permits the survival of haematopoietic progenitors. Br J Haematol. 2001; 112:438–448. [PubMed: 11167845]
33. Taichman RS, Reilly MJ, Verma RS, Emerson SG. Augmented production of interleukin-6 by normal human osteoblasts in response to CD34+ hematopoietic bone marrow cells *in vitro*. Blood. 1997; 89:1165–1172. [PubMed: 9028938]
34. Taichman RS, Reilly MJ, Matthews LS. Human osteoblast-like cells and osteosarcoma cell lines synthesize macrophage inhibitory protein 1alpha in response to interleukin 1beta and tumour necrosis factor alpha stimulation *in vitro*. Br J Haematol. 2000; 108:275–283. [PubMed: 10691855]
35. Zhu J, Garrett R, Jung Y, Zhang Y, Kim N, Wang J, et al. Osteoblasts support B-lymphocyte commitment and differentiation from hematopoietic stem cells. Blood. 2007; 109:3706–3712. [PubMed: 17227831]
36. Crean SM, Meneski JP, Hullinger TG, Reilly MJ, DeBoever EH, Taichman RS. N-linked sialyated sugar receptors support haematopoietic cell-osteoblast adhesions. Br J Haematol. 2004; 124:534–546. [PubMed: 14984505]
37. Ponomaryov T, Peled A, Petit I, Taichman RS, Habler L, Sandbank J, et al. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. J Clin Invest. 2000; 106:1331–1339. [PubMed: 11104786]
38. Jung Y, Wang J, Schneider A, Sun YX, Koh-Paige AJ, Osman NI, et al. Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. Bone. 2006; 38:497–508. [PubMed: 16337237]
39. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol. 2002; 3:687–694. [PubMed: 12068293]
40. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood. 2006; 107:1761–1767. [PubMed: 16269611]
41. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 1996; 382:635–638. [PubMed: 8757135]
42. Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci USA. 1998; 95:9448–9453. [PubMed: 9689100]
43. Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature. 1998; 393:591–594. [PubMed: 9634237]
44. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 1998; 393:595–599. [PubMed: 9634238]
45. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science. 1999; 283:845–848. [PubMed: 9933168]
46. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006; 25:977–988. [PubMed: 17174120]
47. Jung Y, Wang J, Song J, Shiozawa Y, Havens A, Wang Z, et al. Annexin II expressed by osteoblasts and endothelial cells regulates stem cell adhesion, homing, and engraftment following transplantation. Blood. 2007; 110:82–90. [PubMed: 17360942]

48. Nilsson SK, Johnston HM, Whitty GA, Williams B, Webb RJ, Denhardt DT, et al. Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood. 2005; 106:1232–1239. [PubMed: 15845900]
49. Stier S, Ko Y, Forkert R, Lutz C, Neuhaus T, Grunewald E, et al. Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. J Exp Med. 2005; 201:1781–1791. [PubMed: 15928197]
50. Jung Y, Wang J, Havens A, Sun Y, Jin T, Taichman RS. Cell-to-cell contact is critical for the survival of hematopoietic progenitor cells on osteoblasts. Cytokine. 2005; 32:155–162. [PubMed: 16256361]
51. Kiel MJ, Radice GL, Morrison SJ. Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. Cell Stem Cell. 2007; 1:204–217. [PubMed: 18371351]
52. Driessen RL, Johnston HM, Nilsson SK. Membrane-bound stem cell factor is a key regulator in the initial lodgment of stem cells within the endosteal marrow region. Exp Hematol. 2003; 31:1284–1291. [PubMed: 14662336]
53. Adams GB, Chabner KT, Alley IR, Olson DP, Szczepiorkowski ZM, Poznansky MC, et al. Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. Nature. 2006; 439:599–603. [PubMed: 16382241]
54. Wilson A, Murphy MJ, Oskarsson T, Kaloulis K, Bettess MD, Oser GM, et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev. 2004; 18:2747–2763. [PubMed: 15545632]
55. Avecilla ST, Hattori K, Heissig B, Tejada R, Liao F, Shido K, et al. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nat Med. 2004; 10:64–71. [PubMed: 14702636]
56. Yin T, Li L. The stem cell niches in bone. J Clin Invest. 2006; 116:1195–1201. [PubMed: 16670760]
57. Castro-Malaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, et al. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. Blood. 1980; 56:289–301. [PubMed: 6994839]
58. Beresford JN. Osteogenic stem cells and the stromal system of bone and marrow. Clin Orthop. 1989; 240:270–280.
59. Cassiede P, Dennis JE, Ma F, Caplan AI. Osteochondrogenic potential of marrow mesenchymal progenitor cells exposed to TGF-beta 1 or PDGF-BB as assayed *in vivo* and *in vitro*. J Bone Miner Res. 1996; 11:1264–1273. [PubMed: 8864901]
60. Chen JL, Hunt P, McElvain M, Black T, Kaufman S, Choi ES. Osteoblast precursor cells are found in CD34+ cells from human bone marrow. Stem Cells. 1997; 15:368–377. [PubMed: 9323800]
61. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physiol. 1998; 176:57–66. [PubMed: 9618145]
62. Eipers PG, Kale S, Taichman RS, Pipia GG, Swords NA, Mann KG, et al. Bone marrow accessory cells regulate human bone precursor cell development. Exp Hematol. 2000; 28:815–825. [PubMed: 10907643]
63. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002; 418:41–49. [PubMed: 12077603]
64. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. J Cell Biol. 2001; 153:1133–1140. [PubMed: 11381097]
65. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science. 1997; 276:71–74. [PubMed: 9082988]
66. Bianco P, Robey PG. Stem cells in tissue engineering. Nature. 2001; 414:118–121. [PubMed: 11689957]
67. Gronthos S, Zannettino AC, Hay SJ, Shi S, Graves SE, Kortesidis A, et al. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. J Cell Sci. 2003; 116(Part 9):1827–1835. [PubMed: 12665563]

68. Smith JR, Pochampally R, Perry A, Hsu SC, Prockop DJ. Isolation of a highly clonogenic and multipotential subfraction of adult stem cells from bone marrow stroma. Stem Cells. 2004; 22:823–831. [PubMed: 15342946]

69. Falla N, Van V, Bierkens J, Borremans B, Schoeters G, Van Gorp U. Characterization of a 5-fluorouracil-enriched osteoprogenitor population of the murine bone marrow. Blood. 1993; 82:3580–3591. [PubMed: 8260697]

70. Baksh D, Yao R, Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells. 2007; 25:1384–1392. [PubMed: 17332507]

71. Wang Z, Song J, Taichman RS, Krebsbach PH. Ablation of proliferating marrow with 5-fluorouracil allows partial purification of mesenchymal stem cells. Stem Cells. 2006; 24:1573–1582. [PubMed: 16769762]

72. Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, et al. A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia. 2006; 20:857–869. [PubMed: 16498386]

73. Kucia M, Zuba-Surma EK, Wysoczynski M, Wu W, Ratajczak J, Machalinski B, et al. Adult marrow-derived very small embryonic-like stem cells and tissue engineering. Expert Opin Biol Ther. 2007; 7:1499–1514. [PubMed: 17916043]

74. Kucia M, Ratajczak J, Reca R, Janowska-Wieczorek A, Ratajczak MZ. Tissue-specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury. Blood Cells Mol Dis. 2004; 32:52–57. [PubMed: 14757413]

75. Ratajczak MZ, Kucia M, Reca R, Majka M, Janowska-Wieczorek A, Ratajczak J. Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells 'hide out' in the bone marrow. Leukemia. 2004; 18:29–40. [PubMed: 14586476]

76. Kucia M, Wu W, Ratajczak MZ. Bone marrow-derived very small embryonic-like stem cells: their developmental origin and biological significance. Dev Dyn. 2007; 236:3309–3320. [PubMed: 17497671]

77. Ratajczak MZ, Machalinski B, Wojakowski W, Ratajczak J, Kucia M. A hypothesis for an embryonic origin of pluripotent Oct-4(+) stem cells in adult bone marrow and other tissues. Leukemia. 2007; 21:860–867. [PubMed: 17344915]

78. Ratajczak J, Kucia M, Zuba-Surma E, Reca R, Ratajczak MZ. The CD45(−)lin(−) adult marrow-derived CXCR4(+) SSEA-1(+) Oct-4(+) very small embryonic-like (VSEL) stem cells form *in vitro* spheres which may differentiate into CD45(+) hematopoietic cells. Blood. 2006; 108:86A.

79. D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC. Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci. 2004; 117(Part 14):2971–2981. [PubMed: 15173316]

80. Devine SM, Hoffman R. Role of mesenchymal stem cells in hematopoietic stem cell transplantation. Curr Opin Hematol. 2000; 7:358–363. [PubMed: 11055509]

81. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant. 2005; 11:389–398. [PubMed: 15846293]

82. Ball LM, Bernardo ME, Roelofs H, Lankester A, Cometa A, Egeler RM, et al. Cotransplantation of *ex vivo* expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. Blood. 2007; 110:2764–2767. [PubMed: 17638847]

83. Kadereit S, Deeds LS, Haynesworth SE, Koc ON, Kozik MM, Szekely E, et al. Expansion of LTC-ICs and maintenance of p21 and BCL-2 expression in cord blood CD34(+)/CD38(−) early progenitors cultured over human MSCs as a feeder layer. Stem Cells. 2002; 20:573–582. [PubMed: 12456965]

84. Koh SH, Choi HS, Park ES, Kang HJ, Ahn HS, Shin HY. Co-culture of human CD34+ cells with mesenchymal stem cells increases the survival of CD34+ cells against the 5-aza-deoxycytidine- or

trichostatin A-induced cell death. Biochem Biophys Res Commun. 2005; 329:1039–1045. [PubMed: 15752760]

85. Li N, Feugier P, Serrurrier B, Latger-Cannard V, Lesesve JF, Stoltz JF, et al. Human mesenchymal stem cells improve *ex vivo* expansion of adult human CD34+ peripheral blood progenitor cells and decrease their allostimulatory capacity. Exp Hematol. 2007; 35:507–515. [PubMed: 17309831]
86. Pedemonte E, Benvenuto F, Casazza S, Mancardi G, Oksenberg JR, Uccelli A, et al. The molecular signature of therapeutic mesenchymal stem cells exposes the architecture of the hematopoietic stem cell niche synapse. BMC Genomics. 2007; 8:65. [PubMed: 17341312]
87. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol. 2000; 28:875–884. [PubMed: 10989188]
88. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood. 2005; 105:2821–2827. [PubMed: 15591115]
89. Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, Pistoia V, et al. Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells. 2007; 25:1753–1760. [PubMed: 17395776]
90. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. Blood. 2006; 107:367–372. [PubMed: 16141348]
91. Maccario R, Podesta M, Moretta A, Cometa A, Comoli P, Montagna D, et al. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica. 2005; 90:516–525. [PubMed: 15820948]
92. Ramasamy R, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation. 2007; 83:71–76. [PubMed: 17220794]
93. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007; 449:557–563. [PubMed: 17914389]
94. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997; 3:730–737. [PubMed: 9212098]
95. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, et al. Molecular definition of breast tumor heterogeneity. Cancer Cell. 2007; 11:259–273. [PubMed: 17349583]
96. Keller ET, Zhang J, Cooper CR, Smith PC, McCauley LK, Pienta KJ, et al. Prostate carcinoma skeletal metastases: cross-talk between tumor and bone. Cancer Metastasis Rev. 2001; 20:333–349. [PubMed: 12085970]
97. Wang J, Sun Y, Song W, Nor JE, Wang CY, Taichman RS. Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. Cell Signal. 2005; 17:1578–1592. [PubMed: 16005185]
98. Dai J, Kitagawa Y, Zhang J, Yao Z, Mizokami A, Cheng S, et al. Vascular endothelial growth factor contributes to the prostate cancer-induced osteoblast differentiation mediated by bone morphogenetic protein. Cancer Res. 2004; 64:994–999. [PubMed: 14871830]
99. Hullinger TG, Taichman RS, Linseman DA, Somerman MJ. Secretory products from PC-3 and MCF-7 tumor cell lines upregulate osteopontin in MC3T3-E1 cells. J Cell Biochem. 2000; 78:607–616. [PubMed: 10861858]
100. Ravandi F, Estrov Z. Eradication of leukemia stem cells as a new goal of therapy in leukemia. Clin Cancer Res. 2006; 12:340–344. [PubMed: 16428470]
101. Mudry RE, Fortney JE, York T, Hall BM, Gibson LF. Stromal cells regulate survival of B-lineage leukemic cells during chemotherapy. Blood. 2000; 96:1926–1932. [PubMed: 10961896]
102. Iwamoto S, Mihara K, Downing JR, Pui CH, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase. J Clin Invest. 2007; 117:1049–1057. [PubMed: 17380207]
103. Nefedova Y, Landowski TH, Dalton WS. Bone marrow stromal-derived soluble factors and direct cell contact contribute to de novo drug resistance of myeloma cells by distinct mechanisms. Leukemia. 2003; 17:1175–1182. [PubMed: 12764386]

104. Gazitt Y. Homing and mobilization of hematopoietic stem cells and hematopoietic cancer cells are mirror image processes, utilizing similar signaling pathways and occurring concurrently: circulating cancer cells constitute an ideal target for concurrent treatment with chemotherapy and antilineage-specific antibodies. Leukemia. 2004; 18:1–10. [PubMed: 14574330]

105. Vincent T, Mechti N. Extracellular matrix in bone marrow can mediate drug resistance in myeloma. Leuk Lymphoma. 2005; 46:803–811. [PubMed: 16019524]

106. Wang L, O'Leary H, Fortney J, Gibson LF. Ph+/VE-cadherin+ identifies a stem cell like population of acute lymphoblastic leukemia sustained by bone marrow niche cells. Blood. 2007; 110:3334–3344. [PubMed: 17638851]

107. Matsunaga T, Takemoto N, Sato T, Takimoto R, Tanaka I, Fujimi A, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. Nat Med. 2003; 9:1158–1165. [PubMed: 12897778]

108. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat Med. 2006; 12:1167–1174. [PubMed: 16998484]

109. Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, et al. *In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature. 2005; 435:969–973. [PubMed: 15959517]

110. Veiga JP, Costa LF, Sallan SE, Nadler LM, Cardoso AA. Leukemia-stimulated bone marrow endothelium promotes leukemia cell survival. Exp Hematol. 2006; 34:610–621. [PubMed: 16647567]

111. Rizo A, Vellenga E, de Haan G, Schuringa JJ. Signaling pathways in self-renewing hematopoietic and leukemic stem cells: do all stem cells need a niche? Hum Mol Genet. 2006; 15:R210–R219. [PubMed: 16987886]

112. Buzzeo MP, Scott EW, Cogle CR. The hunt for cancer-initiating cells: a history stemming from leukemia. Leukemia. 2007; 21:1619–1627. [PubMed: 17541397]

113. Oakley EJ, Van Zant G. Unraveling the complex regulation of stem cells: implications for aging and cancer. Leukemia. 2007; 21:612–621. [PubMed: 17252019]

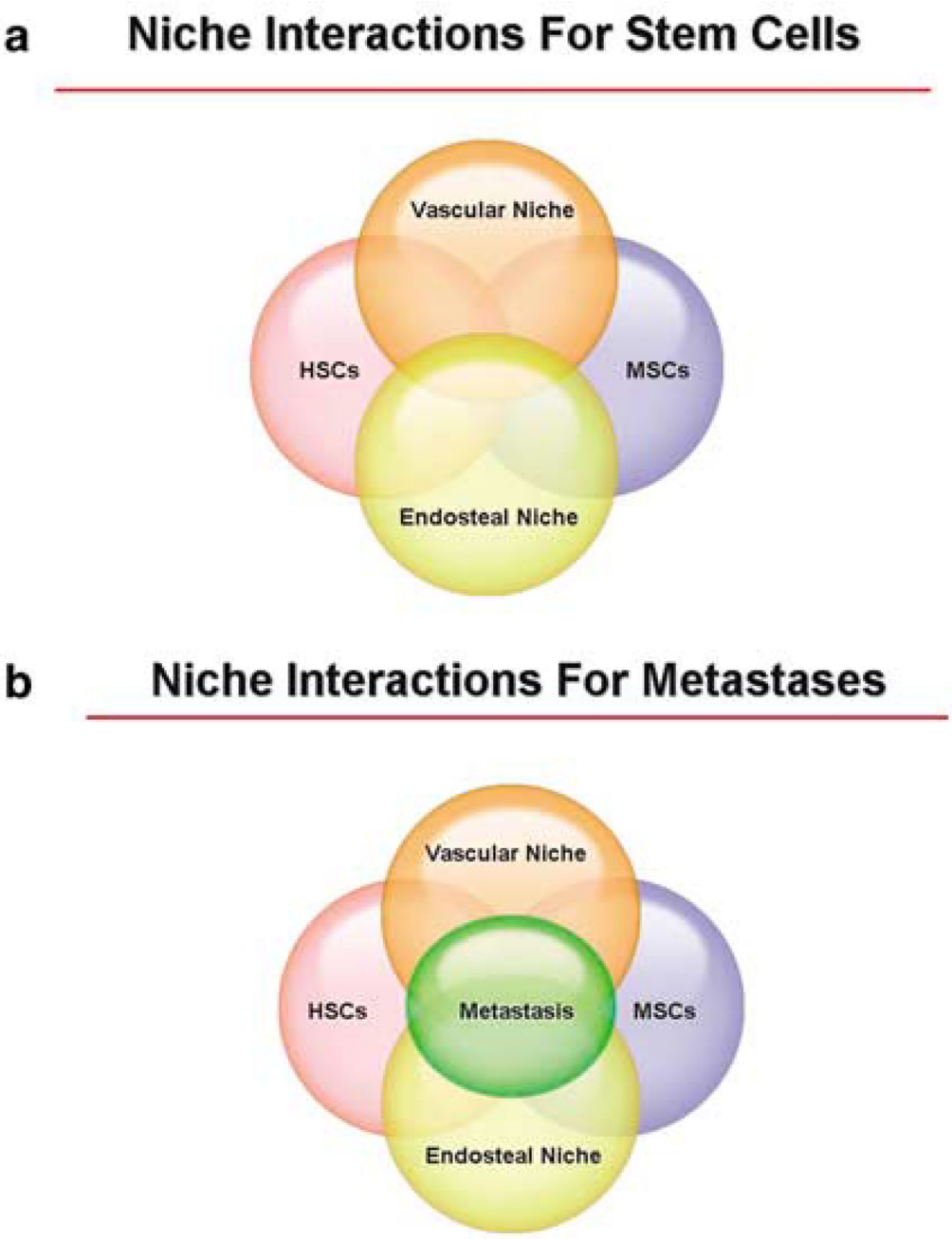

Figure 1.

(**a**) A model of bone marrow niches. In the marrow, osteoblasts and endothelial cells constitute the major cellular components contributing to the endosteal and vascular niches that serve as the microenvironment for maintaining hematopoietic stem cells (HSCs; 'HSC niche'). Osteoblasts and endothelial cells are derived from mesenchymal stem cells (MSCs) and hemangioblasts, respectively. Recent data suggest that MSCs themselves may reside in niches that are in close proximity to the HSC niche. In addition, there is growing evidence that HSCs and MSC co-regulate activities of each other. In the model presented, overlap of HSC, MSC, vascular and endosteal niche function occurs and is required for the coordinated

function of the marrow. (**b**) A model of the neoplastic niche. Increasing evidence suggests that disseminated tumor stem cells reside in niches that facilitate the metastasis and survival of tumors in distant tissues. Much like HSCs and MSCs, the residence of metastatic cells in the niche provides signals that regulate dormancy and escape from chemotherapy and radiotherapy. As such, the residence of metastatic cells in the niche constitutes a molecular parasite of the normal host regulatory functions that exist to supply a constant flux of HSC and MSC progeny throughout the lifetime of the individual.

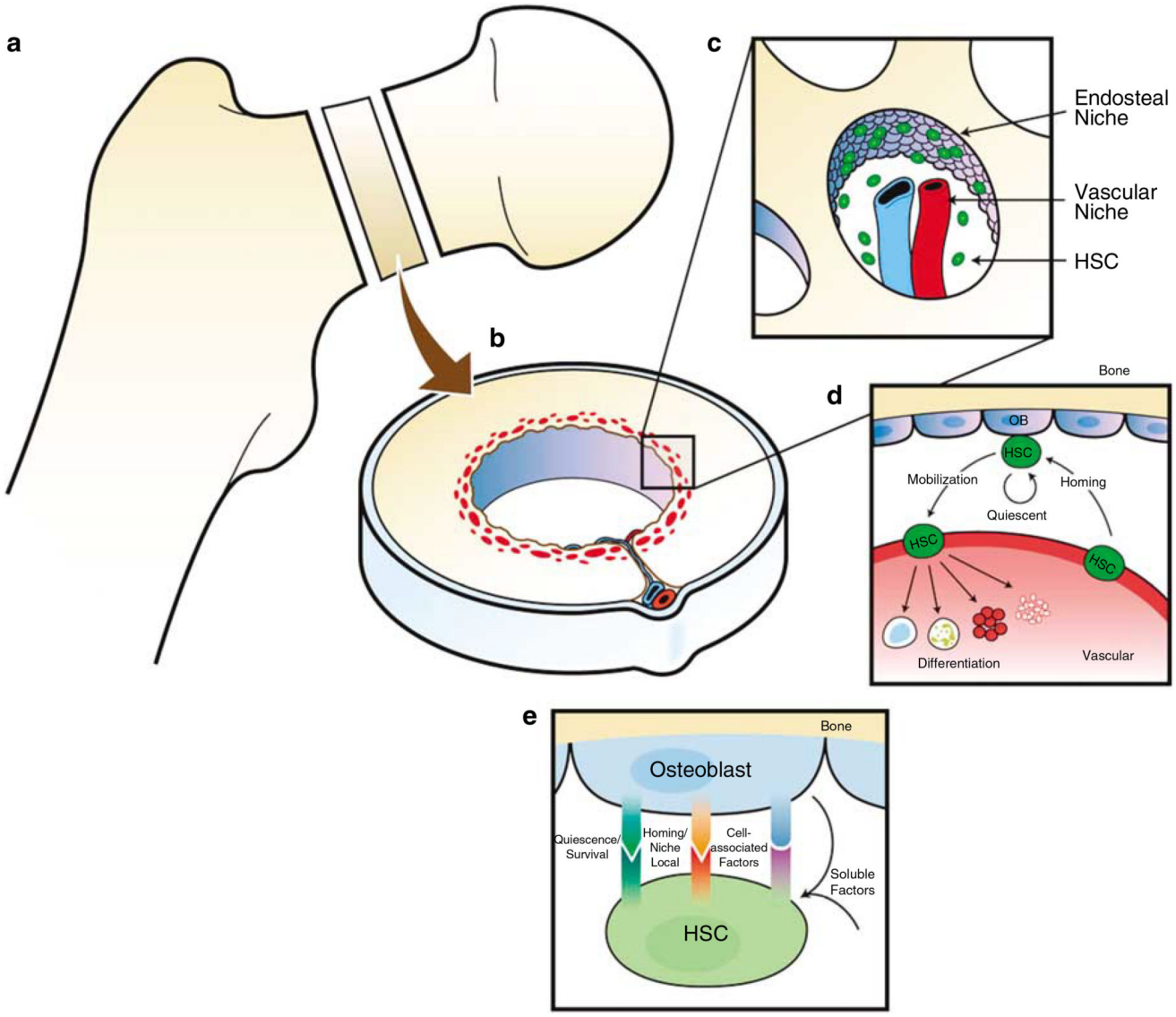

Figure 2.

A model of the hematopoietic stem cell (HSC) niche in long bones. (**a** and **b**) Organization of long bones and location of the HSC niches. Recent studies demonstrate that endosteal osteoblasts and their precursors play a critical role the stem cell 'niche' (**c**). In addition, endothelial cells are likely to contribute to niche function (**c**). Central to these hypotheses are the demonstration of osteoblast (OB)- or endothelial cell-expressed regulatory components that influence stem cell function are likely to include cell-to-cell receptors, and soluble and cell-surface associated cytokines and growth factors. (**d**) Egress into and out of the marrow by HSCs facilitate transit into and out of the vascular niche, which is permissive for proliferation and differentiation. The endosteal niche facilitates HSC maintenance and quiescence. Reciprocal interactions between stem cells and their niches are likely to play key roles in the establishment and maintenance of the stem cell niche in the bone marrow (not shown). (**e**) Factors produced by osteoblasts that influence HSC. Stem cell fate is influenced by specialized microenvironments that remain poorly defined. Osteoblasts produce soluble

hematopoietic-supportive secreted and cell-associated factors that work in concert so that HSCs derive regulatory information from bone, accounting for the localization of hematopoiesis in bone marrow (homing/localization receptors e.g. Annexin II, VCAM-1, CXC chemokine receptor 4 (CXCR4)/CXC chemokine ligand 12 (CXCL12)). Quiescence factors (e.g. bone morphogenic factors, fibroblast growth factors, Flt-3 ligand, Tie2/Ang-1, granulocyte and granulocyte-macrophage colony-stimulating factors, hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF), osteopontin and high levels of extracellular calcium) and cell-associated factors are known to include but are limited to osteopontin, granulocyte and granulocyte-macrophage colony-stimulating factors and transforming growth factors (TGFs). Soluble factors known to influence HSC function include parathyroid hormone and erythropoietin (Epo).

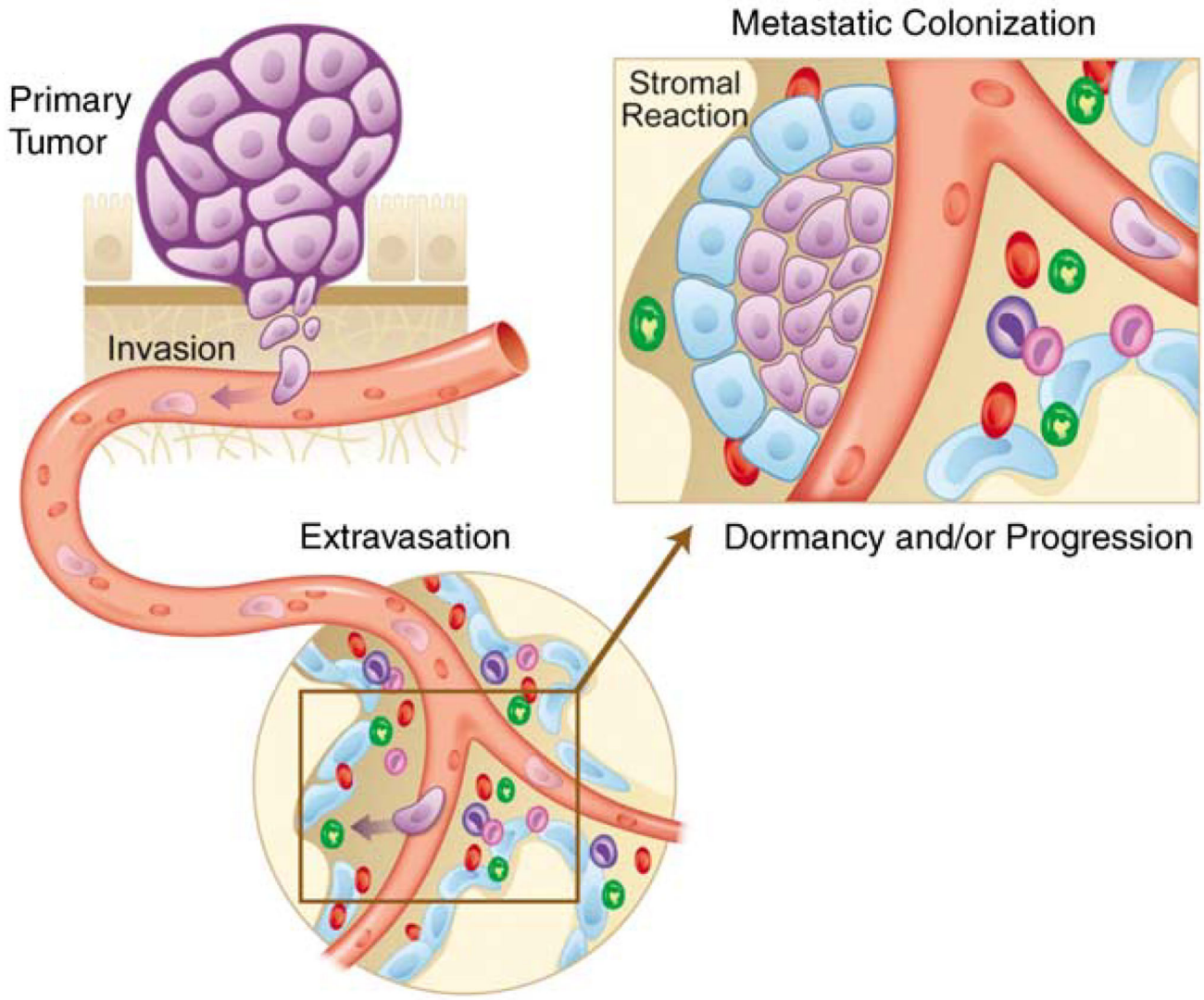

Figure 3.

A model of the neoplastic bone metastasis (this figure was inspired by information found at: http://www.isrec.ch/research/groups/research_groups_detail_eid_1682_lid_2.htm; accessed 30 January 2008). The primary tumor becomes malignant and starts distributing tumor cells through the blood circulation to different organs. Chemokines such as SDF-1/CXC chemokine ligand 12 (CXCL12) may mediate the homing of neoplasm. Circulating neoplasm invades through the endothelial layer and basement membrane and reaches the secondary site or neoplastic niche. Once neoplasm localize to the marrow, those cells that are able to successfully engage the niche regulatory systems may become dormant through specific cell-to-cell adhesive interactions. In the mean time, those cells start grow again at secondary sites.

Table 1

Parallels between hematopoietic stem cells and metastatic neoplasms

Hematopoietic stem cells	Metastatic neoplasms
Hematopoietic stem cells	Leukemic/cancer stem cells
Unipotent	Undifferentiated
Homing	Metastasis
Colonization	Invasion
Quiescence	Dormancy
Migration	Dissemination
Self-renewal	Recurrence
Differentiation	Tumorigenesis

Molecular similarities between HSCs, LSCs and CSCs. The bone marrow stromal cells provide survival signals to HSCs, LSCs and CSCs probably through similar pathways. Yet, although a number of similarities exist between normal HSCs, LSCs and CSCs in terms of molecular mechanisms that regulate self-renewal and interactions with the bone marrow microenvironment, clearly differences exist as well.