

REVIEW

A niche opportunity for stem cell therapeutics

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The success of hematopoietic stem cell (HSC)-based therapies relies on the ability of the stem cells to both engraft and self-renew sufficiently in the bone marrow microenvironment. Previous studies identified that a number of components of bone contribute to the regulation of HSCs indicating that they participate in a stem cell 'niche'. This niche is a dynamic microenvironment that changes during development and with varying physiologic states. Components of it, such as the osteoblast, can be modulated through pharmacological treatment. Reasoning that the stem cell niche may

be manipulated to augment the effectiveness of stem cell therapies, we demonstrated that daily treatment with parathyroid hormone (a clinically approved method for increasing osteoblast function) resulted in therapeutic benefit in three clinically relevant models of stem cell therapy. These results suggest that the niche may be a pharmacological target for altering stem cell function in settings of regenerative medicine.

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Introduction

Hematopoietic stem cells (HSCs) are an effective cell-based therapy due to their unique ability to both self-renew and give rise to all cell lineages of the hematopoietic system for the life of the individual organism. Therefore, a single treatment targeted at the HSCs can achieve life-long therapeutic benefit. An additional advantage of HSCs is that through the use of standard peripheral blood, bone marrow or umbilical cord blood transplantation protocols, these cells can be semi-purified and specifically manipulated *ex vivo*. These characteristics make gene modification of HSCs attractive as a durable means for addressing blood-related disorders.

A number of trials have been performed testing the ability of HSC gene transduction to result in clinical improvements with at least some indications of therapeutic success.¹ However, one limitation is the need for large numbers of cells to be transplanted. Engraftment efficiency is extremely low, rendering the likelihood of successful transplant of transduced cells that are themselves a minority of the transport quite low. Therefore, it appears that strong selective conditions favoring the gene-modified cell are necessary for evidence of engraftment to be detectable.² In many genetic diseases, this selective pressure may not be present, and therefore a better understanding of the mechanisms of engraftment

and self-renewal in the bone marrow microenvironmental niche is required to improve the potential for success.

The hematopoietic stem cell niche

One approach to increase engraftment is to understand and manipulate the mechanisms involved in the expansion of stem cells in their niche. Hematopoiesis lends itself to the study of microenvironmental determinants, in part because of its changing location during mammalian development. Primitive HSCs first arise in the yolk sac, before definitive HSC function is found in the para-aortic splanchnopleura or aorto-gonadal-mesonephros regions of the developing embryo. The liver then becomes invested with stem cells, and becomes the predominant source of hematopoiesis until approximately the second trimester when stem cell transition to the bone marrow takes place.³ With each shift in location there are differences in the proliferation and mature cell production of stem cells suggesting a potential contributing role of the microenvironment.

In 1975, it was identified that adult 'bone marrow cell populations are shown to conform to a well-defined spatial organization.'⁴ In other words, it was demonstrated that the HSCs were specifically localized in very close proximity to the endosteal surface of bone (which has been confirmed more recently using phenotypically isolated HSCs⁵). Following the identification of the localization of the HSCs, it was hypothesized in 1978 that the HSCs must reside in stable, custom micro-environments that control the behavior of the stem cells, specifically enabling their self-renewal.⁶ These regions were termed the stem cell 'niches.' One proposed role of the stem cell niche is to prevent the stem cell from

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becoming accessible to the differentiating influences of the surrounding microenvironment.⁷ The possibility that the HSCs in the bone marrow reside in niches at the endosteal surface of the bone marrow was not further investigated for another 16 years, when Taichman and Emerson⁸ tested whether stem cells were affected by osteoblasts. Their experiments demonstrated that the osteoblasts released cytokines known to affect HSC physiology *in vitro*, and that these cells could be stimulated to increase expression of these cytokines.⁹ In addition, the presence of osteoblasts in the stromal layers enhanced their HSC supportive capacity *in vitro*.¹⁰

In mammalian model systems, the location of stem or precursor populations within numerous solid tissues has been described, but delineating specific associated cells and how they participate in regulating stem cell function has generally been lacking. The use of invertebrate-based models, however, has created particularly productive systems in which to examine the niche context of stem cells. The gonadal tissue from *Drosophila melanogaster* and *Caenorhabditis elegans* has permitted the definition and identification of ancillary niche cells, physical interactions and the molecular pathways that govern the interplay between the stem cell and its local environment.^{11,12} From these studies we hypothesized that the osteoblast could be an essential component of the HSC niche *in vivo*, yet direct evidence for this was lacking. This was due to a lack of *in vivo* models where only the osteoblastic population of cells was specifically altered. For these reasons our group examined a mouse model where the osteoblastic cell population was specifically activated and thus increased in number and activity.¹³ This was achieved through the expression of a constitutively active form of the PTH/parathyroid hormone-related protein receptor specifically in the osteoblastic cells via the mouse $\alpha 1(I)$ collagen promoter. The resulting phenotype in this transgenic mouse was an increased trabecular bone volume in the metaphyses of the long bones. This increase in the osteoblast population number and activity resulted in an increase in the HSC population, whereas the more mature hematopoietic progenitor cells remained unperturbed. An accompanying paper by Zhang and colleagues¹⁴ who specifically increased osteoblast number and activity through conditional inactivation of the BMPRI1A receptor also demonstrated essentially identical results. These studies demonstrated that the osteoblast is a key component of the HSC niche in the bone marrow *in vivo*. The specificity of the effect of the osteoblast on the HSCs also verified the notion that the stem and progenitor cells reside in different micro-environmental niches in the bone marrow, which are regulated by different molecular mechanisms.

Recently it has been proposed that in addition to the endosteal niche of the HSCs, there also exists another location where the HSCs reside in the adult bone marrow, that is, adjacent to blood vessels.¹⁵ This vascular niche was proposed based on the observation that many HSCs are found in the perivascular zone (while a smaller proportion is adjacent to the endosteal surface). However, there is still limited information on whether this perivascular region is a site of self-renewal. Since HSCs are known to regularly traffic into and out of the circulation, there remains the formal possibility that the perivascular localization of cells represents cells impeded in their movement rather than serving as a true

niche. Recent data on CXCL12-expressing 'reticular' cells have indicated that these cells are needed for HSC persistence and are found in both perivascular and endosteal regions.¹⁶ Taken together, the data indicate that there are likely multiple sites where HSCs reside and are regulated. The region we have the most information about in terms of molecular regulation is the endosteal region and that will be the emphasis of this review.

Targeting stem cell homing and engraftment

The developmental process of HSC translocation from the fetal liver to the bone marrow microenvironment via the peripheral circulation reflects the ability of HSCs to find their way to a niche that is preserved in adulthood and has been greatly exploited for the purposes of bone marrow transplantation. The process of hematopoietic engraftment following injection of the stem cells involves the coordinated steps of homing to the bone marrow space via the endothelial-lined sinusoids, lodgment of the stem cells at the endosteal niche, followed by self-renewal and differentiation of the stem cells into all lineages of the hematopoietic system. In the converse situation, HSCs can mobilize into the peripheral circulation through vacating the stem cell niche, followed by interacting with the endothelial lined sinusoids to enter into the peripheral circulation. Many studies have identified components of the HSC homing machinery to the bone marrow (reviewed by Lapidot *et al.*¹⁷). Yet, many of these mechanisms are involved in the homing of all leukocytes and not restricted to the HSC population of cells. However, this has led to pharmacological manipulations of these processes, some of which are currently in clinical use (Figure 1).

In contrast, the mechanisms of lodgment of the stem cells in the endosteal niche, first proposed by Wolf in 1974,¹⁸ are not clear. Given that HSCs specifically reside at the endosteal surface of bone, we reasoned that bone itself or more specifically the mineral content of bone, may provide a lodgment signal for the HSCs. To address this we examined HSCs that lack a receptor known to be influenced by fluctuations in extracellular ionic calcium, the calcium sensing receptor.¹⁹ We demonstrated that cells lacking this receptor are defective in their ability to specifically localize to the endosteal niche and thus engraft in the bone marrow. This provides an, as yet untested, approach to augment or disrupt the interaction

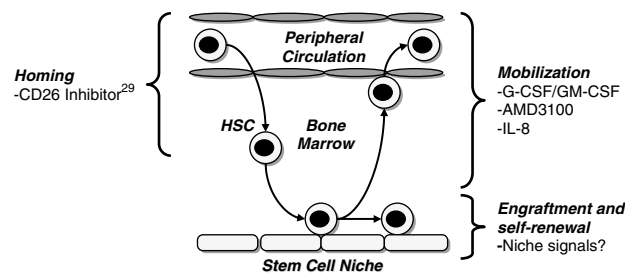


Figure 1 Current and potential pharmacological treatments to enhance hematopoietic stem cell-based therapies. Multiple steps in the processes of stem cell homing, engraftment and mobilization could be targeted using pharmacological treatments.

of HSC localization to the niche for stem cell engraftment or mobilization purposes.

Pharmacological manipulation of the stem cell niche

The observation that the osteoblasts were a key component of the HSC niche and that activation of those cells led to an expansion of the stem cell population offered a possibility for a novel stem cell therapy: targeting the microenvironmental niche rather than direct targeting of the stem cell itself. Reasoning that we could recapitulate the effects observed in our transgenic model of constitutive activation of the PTH/parathyroid hormone-related protein receptor through the addition of exogenous PTH, we examined the effects of PTH *in vitro* and *in vivo*. PTH treatment of stromal cells *in vitro* expanded the osteoblast population and thus was able to augment their support of primitive cells. Daily injection of PTH for 4 weeks similarly expanded the HSC population *in vivo*. However, more impressive results were obtained when we studied bone marrow transplantation with limiting numbers of bone marrow cells. Daily injections of PTH following injection of bone marrow cells increased survival from 27% in the control group to 100% in the PTH-treated group¹³. These data indicate the potential for a relatively modest change in stem cell number resulting in a marked physiologic benefit and suggest that targeting the microenvironment may be a means to alter stem cells in a therapeutic context. Further studies by our group also indicated that PTH treatment could enhance HSC therapy in three clinically relevant therapeutic contexts. Specifically, treatment of mice with PTH was shown to (1) expand resident HSCs, which resulted in an increased number of HSCs being mobilized into the peripheral circulation following standard regimens; (2) expand HSCs delivered to the niche by standard bone marrow transplantation protocols and (3) protect resident stem cells from the myelotoxic effects of chemotherapeutic agents, particularly when used in combination with granulocyte colony-stimulating factor to support neutropenia²⁰ (Figure 2). However, whether

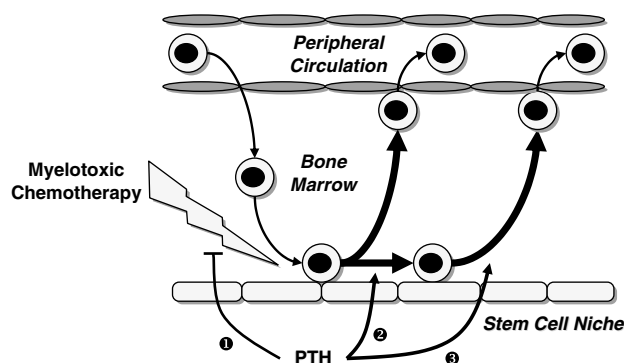


Figure 2 Modes of action of parathyroid hormone (PTH) treatment for hematopoietic stem cell (HSC)-based therapies. Parathyroid hormone has been shown in mice to (1) protect resident stem cells from myelotoxic chemotherapy, (2) increase expansion of stem cells following engraftment of cells in the niche or (3) expand resident stem cells to increase HSCs mobilization into the peripheral circulation with standard mobilizing regimens.

PTH can be a treatment to augment stem cell number or function in humans is presently unknown. rPTH is a drug currently approved for use in humans in the setting of osteoporosis and appears to be a very safe, well-tolerated medication. The potential for translating our studies into a clinical setting is therefore readily testable.

Using PTH to target the osteoblast is but one possible therapeutic approach. Other studies identifying signaling pathways and components of the HSC niche have demonstrated that these pathways too can be manipulated for therapeutic effect (Figure 2). In particular, studies demonstrating the role of the Tie-2 receptor on HSCs also identified that its ligand Ang-1 can improve survival of mice following lethal doses of myelotoxic drugs.²¹ Similar effects were also seen following treatment of mice with hyaluronic acid, a known extracellular component of the bone marrow niche.²² If confirmed in humans, these treatments would offer a new type of approach to stem cell therapies, targeting the niche rather than the stem cell directly. Clinical trials evaluating this concept are currently under development.

Targeting the cancer stem cell niche

Many studies have hypothesized that analogous to normal tissue, most cancers also have a stem cell or tumor-initiating cell.²³ While there is still limited information on the identity of these cells, and even less on the location of the cancer stem cell niche, it has been suggested that targeting the microenvironment of these cells could also result in a reduction of the tumor burden.²⁴ This has been shown to be the case in brain tumors, where it is thought that similar to neural stem cells, the tumor stem cells reside in vascular niches. Therefore, disruption of the vascular network within the tumor not only reduces the tumor size due to restriction of blood flow to the tumor, but may also disrupt the stem cell niche and therefore eliminate these cells.²⁵ This approach may ultimately provide a complement to therapies targeting the cancer cells themselves.

With respect to hematological malignancies, many malignant cell types are known to metastasize or have their primary tumor site in the bone marrow microenvironment. This too has led to many studies proposing the idea that the microenvironment surrounding the malignant cell can be targeted to eradicate the tumor. This therapeutic strategy has been particularly effective in settings such as multiple myeloma where pharmacological agents are not only toxic to the tumor cells, but also decrease the adhesion of the tumor cells to their respective microenvironment.²⁶ This ultimately leads to the disruption of the proliferation of the tumor cells, and thus reduces the tumor burden. The idea that there are leukemic stem cells and that they do exist in a niche has been proposed from a small number of studies. These studies have identified that leukemic stem cells preferentially use the adhesion molecule CD44 to establish themselves in the bone marrow.^{27,28} Blocking of this receptor with monoclonal antibodies has led to a reduction in the number of leukemic cells in mice. Therefore, CD44 is a key molecule in the interaction of leukemic stem cells with their niche and thus suggests a means of intervention quite distinct from usual cytotoxic chemotherapies.

Additionally, these studies showed that normal stem cells were unaffected, thereby pointing to molecular distinctions between normal and malignant cell interactions with their microenvironments. Whether such distinctions can be further identified and provide a therapeutic window or whether the shared features of the niche(s) will compromise a niche-based approach remains to be determined. Indeed, whether normal and leukemic stem cells have different niches or compete for the same niche is still an open question and one that warrants further investigation.

Summary

The reductionist approach to stem cells, focusing on the cells in isolation or on cell autonomous regulators, has been extremely useful in revealing the identity of the cells and key determinants of their function. However, investigating the cells in the complex microenvironment in which they reside offers new possibilities for understanding and manipulating them. Defining the osteoblast as a component of the stem cell niche permitted targeting of the osteoblast function to affect stem cell function. The result was a marked improvement in HSC engraftment, and thus animal survival after transplantation that supports the general strategy of manipulating the microenvironment to alter stem cell fate. Therefore, defining the niche is of biological interest and may have practical utility in designing therapies to enhance stem cell number or function. Pursuit of niche components in other tissues will be of considerable interest to determine the broad applicability of this approach to tissue regeneration. Regardless of their success, however, defining niche constituents provides insight into a larger, complex system that can be singly evaluated and manipulated to gain a fuller understanding of the interactions that govern physiological stem cell control.

Conflict of interest

DTS is a consultant for shareholder in Fate Therapeutics, Inc.

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