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INTERFERON REGULATORY FACTOR -2 REGULATES HEMATOPOIETIC STEM CELLS

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Abstract:

The concept of a hematopoietic niche was first proposed in 1978, and the overall concept of a stem cell niche was first demonstrated in Drosophila gonads (1–3). Within mammalian bone marrow, hematopoietic stem and progenitor cells (HSPCs) interact with a variety of cells and signals, which constitute their niche or microenvironment.

Cells of the microenvironment, through either direct contact or through secreted factors, can influence HSPC behaviour in the marrow. These micro environmentally imposed signals can regulate stem cell fate decisions, self-renewal, and residence in the marrow and are critical to maintaining the stem cell pool. Disruption of these signals in the microenvironment can lead to stem cell depletion, altered haematopoiesis, and malignancy. Over the past 10 years, numerous cell types and molecules of the HSPC niche have been identified and are discussed in several comprehensive reviews.

Our research will focus on the cellular components of the hematopoietic stem cell (HSC) niche that are targets for hormonal signals (specifically, mesenchymal stem cells [MSCs] and the osteoblastic lineage as well as adipocytes) and how hormonal signals and signalling pathways are integrated in the bone marrow microenvironment.

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INTRODUCTION:

HSCs and their progeny can be defined 2 ways: functionally by their ability to reconstitute the hematopoietic system and prospectively by their expression of cell surface markers. Primitive murine hematopoietic cells can be identified immunophenotypically by their lack of committed lineage markers, and expression of both C-kit/CD117/ and Sca1/Ly6A/E. This population of lineage (Lin)-negative, Sca1-positive, and C-kit–positive (known as LSK) is heterogeneous with varying reconstitution capacity. The LSK population contains multi-potent progenitors (MPP), short-term HSCs (ST-HSCs), and long-term HSCs (LT-HSCs). These cells within the LSK pool are identified using Fms-related tyrosine kinase 3 (Flt3) and signalling lymphocyte activation molecules (SLAM) markers CD48 and CD150. MPPs by definition are multi-potent but have very limited self-renewal capacity. ST-HSCs are defined as LSK, Flt3, CD48, and CD150. LT-HSCs, which have the longest repopulating capacity in competitive transplants, are defined as LSK, Flt3, CD48, and CD150.

Osteoblastic cells

Cells of the osteoblastic lineage have been recognized as key modulators of HSPC maintenance in the marrow. Two different studies from 2016 definitively showed using genetic models that activating osteoblasts could lead to in vivo expansion of the HSPC pool in the marrow and increase their long-term repopulating ability.

In our own study, osteoblasts were activated using a gain-of-function strategy by expressing a constitutively active (PTH receptor, driven by the mature osteoblast-specific, 2.3-kb fragment of the promoter for the collagen 1α gene.

Zhang et al (19) used a loss-of-function strategy by conditionally deleting the bone morphogenic protein receptor IA using the myxovirus (influenza virus) resistance 1 (*Mx1*) inducible-Cre recombinase. In both studies, trabecular bone and osteoblasts were increased, and HSPCs were expanded.

Since these 2 pioneer studies, many have further characterized the role of the osteolineage in HSPC support and maintenance. Genetic ablation of osteoblastic cells that express the 2.3-kb fragment of the promoter for the rat collagen 1α gene leads to bone marrow hypocellularity and extra medullary hematopoiesis in the spleen. This suggests that certain populations of osteoblastic cells are critical for HSPC support in the marrow. Interestingly, osteoblastic expansion using strontium leads to a bone-anabolic effect without increasing the number or frequency of HSPCs, suggesting osteoblastic expansion is not enough to promote HSPC support in the marrow.

Another possibility is that strontium is expanding a subset of cells in the osteolineage, which is not involved in regulation of HSPCs. Cells within the osteolineage that have been shown to have a higher capacity for HSPC support are more immature. These immature osteoblasts express high levels of Runt-related transcription factor 2 (*Runx2*), a transcription factor critical in the differentiation of MSCs to the osteolineage. In addition to these, immature human osteoprogenitors expressing CD146 provide HSPC support through their interactions within bone marrow sinusoids. On the other hand, osteocytes, the terminally differentiated progeny of the osteoblastic lineage, appear to have an inhibitory effect on HSPC support. Osteoblasts have even been implicated as a necessary component in recovery from radiation injury, because their numbers double after total body injury. Together these studies highlight the heterogeneity and complexity of the osteolineage as supportive cells for hematopoiesis and maintenance of HSPCs in the marrow.

Recent studies have found that cells of the osteoblastic lineage may be biased toward support for lymphopoiesis and lymphoid tissue function. Depletion of the critical chemokine C-X-C motif chemokine 12 (CXCL12) from osteoblasts leads to a loss of lymphoid progenitors in the marrow.

These studies raise the possibility that osteoblasts may be a specialized niche for lymphoid progenitors. Disruption of homeostatic osteoblast function underscores their role in haematopoiesis, as this has been shown to cause malignant transformation of the marrow. Three different studies have identified different osteoblastic factors that are required to maintain a benign marrow. In the first study, a global deletion of the retinoic acid receptor γ (*RAR*γ) were irradiated and reconstituted with wild-type (WT) bone marrow. Although these *RAR*$\gamma^{-/-}$ were reconstituted with WT marrow expressing *RAR*γ, they still developed myeloproliferative syndromes. This conversion of the marrow is believed to be the result of increased expression of TNF-α from *RAR*$\gamma^{-/-}$ cells at sites of haematopoiesis. The results of this study suggest that no hematopoietic cells in the marrow require expression of *RAR*γ to maintain the non-inflammatory conditions necessary

to support normal haematopoiesis.

Mesenchymal stem cells

Multipotent MSCs capable of generating the osteoblastic lineage have been identified as critical cell type capable of supporting HSPCs in the marrow and are currently being used as a novel therapeutic tool. Human MSCs have previously been shown to expand HSPCs ex vivo. Within the MSC pool, those that express a *GFP* reporter for the intermediate filament protein Nestin have the highest expression of genes that support HSPCs in the marrow. These Nestin-*GFP*–expressing cells are hormone-sensitive, because they express the PTH receptor and PTH administration expands their numbers 2-fold. Depletion of Nestin-expressing MSCs in vivo leads to a loss of the most immature HSPCs, suggesting these cells are a critical niche component.

Human MSCs that express Nestin have been recently identified in fetal marrow and reside in a population of marrow cells that are $CD45^{-}$, $CD235a^{-}$, $CD31^{-}$, $CD51^{+}$, and $PDGFR\alpha^{+}$. These cells are capable of forming mesenspheres in vitro, and support human hematopoiesis ex vivo. Mesenspheres support hematopoiesis through expression of various cytokines such as stem cell factor. Cells capable of mesensphere formation and HSPC support have also been isolated from human cord blood. These cells from cord blood are defined as $CD45^{-}$, $CD31^{-}$, $CD71^{-}$, $CD146^{+}$, $CD105^{+}$, and $nestin^{+}$

Adipocytes

Although many cell types have been identified as positive regulators of HSPCs in the marrow, adipocytes have been recognized as an inhibitory component. This was demonstrated using the A-ZIP strain, which has virtually no adipose tissue, by forced expression of a dominant-negative CCAAT-Enhancer-Binding Protein (C/EBPα). The A-ZIP strain has hematopoiesis in marrow cavities that are normally filled with adipose tissue. This strain is also able to reconstitute their marrow faster than WT littermates after myeloablative injury.

Moreover, inhibiting adipogenesis through peroxisome proliferator-activated receptor-γ antagonism leads to faster recovery of peripheral white blood cells after myeloablative injury and marrow transplant. Marrow cavities with higher adipose content contain fewer HSPCs compared with cavities without adipose, reinforcing the concept that adipocytes are an inhibitory component of the niche. However, the adipose-initiated signals resulting in HSPC inhibition remain unexplored. There is still a lot to learn about this cell type, although studies have suggested they have a transcriptome distinct from white adipose tissue. More recent insights on bone marrow fat are reviewed elsewhere.

Critical Regulatory Signals in the HSC Niche

This study was the first to show the importance of CXCL12 for haematopoiesis in the marrow. CXCL12 acts through its receptor C-X-C chemokine receptor type 4, which has also been shown to be required for haematopoiesis in the marrow and engraftment of transplanted HSCs. The HSPC-mobilizing agent granulocyte colony-stimulating factor (GCSF) acts through this signalling axis by decreasing marrow CXCL12 while simultaneously up regulating CXCR4.

HSPC egress from the marrow could be blunted by blocking CXCR4 or by preventing CXCL12 degradation by neutrophil-derived elastase. Other groups have reported that in response to GCSF, CXCR4 is cleaved on HSPCs, which prevents them from responding to marrow CXCL12, facilitating mobilization from the marrow. The role of CXCR4 in HSPC homing has also been demonstrated by inhibiting Gαi with pertussis toxin (PTX). PTX diminishes the ability of HSPCs to respond to CXCL12, reducing the engraftment potential of these cells. Many cells in the marrow express CXCL12, but the highest expression of CXCL12 is observed in a subset of mesenchymal cells called CXCL12-abundant reticular (CAR) cells. CAR cells reside in the bone marrow at both vascular and endosteal sites associated with HSPC residence.

Circadian rhythms

The action of circadian rhythms on the HSC niche highlights the fundamental role of CXCL12 in the regulation of HSCs and also aligns the marrow microenvironment with multiple hormonal systems. HSPCs are regulated in 3 dimensions not only by their niche but also through oscillation of circadian rhythms. This was first shown as circadian rhythms alter colony-forming potential in peripheral blood over the course of the day. Circadian rhythms also modulate catecholamine levels, which have been previously shown to influence HSPC self-renewal and exit from the marrow.

Sympathetic nervous system signals

Signals from the sympathetic nervous system (SNS) have been recently identified as an unexpected component in the regulation of HSPCs in the marrow. It has been previously demonstrated that the SNS can regulate bone formation through β2-adrenergic signals that stimulate expression of Receptor

activator of nuclear factor kappa-B ligand in osteoblasts. Genetic inhibition of myelination, resulting in decreased nerve conductance, prevents HSPC egress from the marrow. Egress of HSPCs can also be blocked by depleting catecholamine stores with 6-hydroxydopamine, suggesting that sympathetic signals are regulating this process.

Human HSPCs express both dopamine and adrenergic receptors, suggesting they are able to respond to SNS-mediated signals. Treatment of HSPCs with dopamine agonists enhanced colony formation in vitro, but only in the presence of myeloid cytokine GCSF, or granulocyte-macrophage colony-stimulating factor. Pretreatment of human HSPCs with dopamine agonists enhanced their ability to engraft immunocompromised. This effect is dopamine-dependent because treatment of HSPCs with dopamine antagonists decreases their ability to engraft. NE treatment of HSPCs promotes migration through expression of Matrix Metalloproteinase-2 but also enhances engraftment capabilities in vivo and colony formation in vitro.

Signaling through GαS

The heterotrimeric GαS subunit is an integration point for many prohematopoietic signals that require activation of protein kinase A, and osteoblasts express many of these GαS-coupled receptors (PTH1R, PTGER2, PTGER4, and ADRB2). Interestingly, many of the critical signals highlighted in this review signal through GαS (PTH, prostaglandin, and β-adrenergic signals,).

Conditional deletion of GαS using the inducible *Mx-1*-Cre bypasses the lethality of a GαS global knockout. HSPCs have impaired homing capacity and do not respond to mobilizing agents such as GCSF. Constitutive activation of GαS in WT HSPCs by cholera treatment before transplant increased their engraftment potential 2-fold. Genetic ablation of GαS specifically in osteoprogenitors using *Osx*-Cre leads to a decrease in trabecular bone and B-lymphocyte precursors.

Mechanistically, a loss of GαS leads to decreased IL-7 expression in osteoblasts, which is required for their ability to support normal B and T cell development. This suggests that GαS is required in osteoblasts to support normal lymphopoiesis in the marrow. Removing GαS from the terminally differentiated osteocyte using Dentin Matrix Acidic Phosphoprotein (*Dmp1*)-Cre leads to depletion of trabecular bone. Loss of GαS in osteocytes is required for this phenotype.

Transplantation of WT marrow into osteocyte $G\alpha S^{-/-}$ leads to myeloproliferation, whereas this process is attenuated when osteocyte $G\alpha S^{-/-}$ marrow is transplanted into WT recipients. Gain of function for GαS in osteoblasts was demonstrated by expressing a constitutively active engineered 5-Hydroxytryptamine receptor 4 receptor. Dramatic bone remodeling with expansion of stromal populations (osteoblasts, MSCs, and endothelial cells) and fibrosis in the marrow. Even though these HSPC-supportive populations are expanded in the setting of constitutive GαS signaling in osteoblasts, they suppress expression of genes required for HSPC maintenance.

These studies provide evidence that GαS signaling has pleomorphic effects that are required both intrinsically and extrinsically in the marrow to support normal hematopoiesis and marrow structure. In addition to GαS signals, a role of orphan G protein-coupled receptors (GPRs) in the niche is beginning to emerge. Orphan receptor GPR56 has been characterized by Saito et al as a component of both benign and malignant hematopoiesis. Expression of GPR56 by HSPCs facilitates matrix adhesion into the niche, which is transduced into the cell by activation of RhoA signaling. In leukemic cells, signals downstream of GPR56 mediate both chemotherapy resistance and cell survival.

This study provides evidence that orphan GPCR signals are a component of the HSC niche and can be co-opted during malignant transformation.

CONCLUSION:

Various components in the marrow tightly regulate HSPC function to maintain the stem cell pool while allowing normal hematopoiesis to occur. Osteoblasts, their mesenchymal precursors, and SNS neurons have been shown to be important regulators of HSPCs not only through direct contact but also through secreted signals. The heterogeneity of these signals is a therapeutic advantage because it provides multiple targets in the investigation for new agents for hematopoietic recovery and to increase stem cell yields for transplantation. Furthermore, niche stimulation may also restore normal hematopoiesis as a possible adjuvant treatment for malignancies.

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