

Chapter 2

Hematopoiesis

Hematopoietic cells, or hemopoietic cells, represent bone marrow-derived cell types that circulate in blood (including mature cell types and their precursors). Hematopoietic cells are categorized into *myeloid cells* (basophils, eosinophils, neutrophils, erythrocytes, thrombocytes, monocytes and macrophages, and mastocytes) and *lymphoid cells* (B lymphocytes, various types of T lymphocytes, which are the only hematopoietic cells that can be generated elsewhere than in the bone marrow, and natural killer [NK] cells, that are cytotoxic lymphocytes also called large granular lymphocytes).

Stromal cells is another collective term for different cell types in a given tissue at a given time that do not pertain to functional components of this tissue, i.e., *parenchymal cells*. In particular, the bone marrow stroma yields a hematopoietic microenvironment that consists of various cell types, such as mesenchymal progenitors, fibroblasts, adipocytes, monocytes and macrophages, endothelial and smooth muscle cells, T-lymphocytes, among others. These cells can support short- and long-term hematopoiesis via membrane-bound and secreted hematopoietic factors that operate on hematopoietic stem and progenitor cells.

Hematopoietic stem cells produce by mitosis either stem cells of the same type (self-renewal) or progenitors leading to precursor, immature, and mature blood cells. In steady state, most stem cells are dormant, whereas few are active [37]. Hematopoietic stem cell self-renewal is regulated by intrinsic and extrinsic signals.

Blood-cell progenitors and mature cells of the granulocytic, monocytic, megakaryocytic, and erythroid lineages can be generated from differentiated cells. For example, human dermal fibroblasts that bypass the pluripotent stem cell state can give rise to multipotent hematopoietic progenitors. These fibroblasts synthesize predominantly octamer-binding transcription factor Oct4, or POU domain, class-5, homeodomain-containing transcription factor POU5F1, as well as PTPRc phosphatase, or panhematopoietic marker CD45, during the multipotent reprogramming [38].

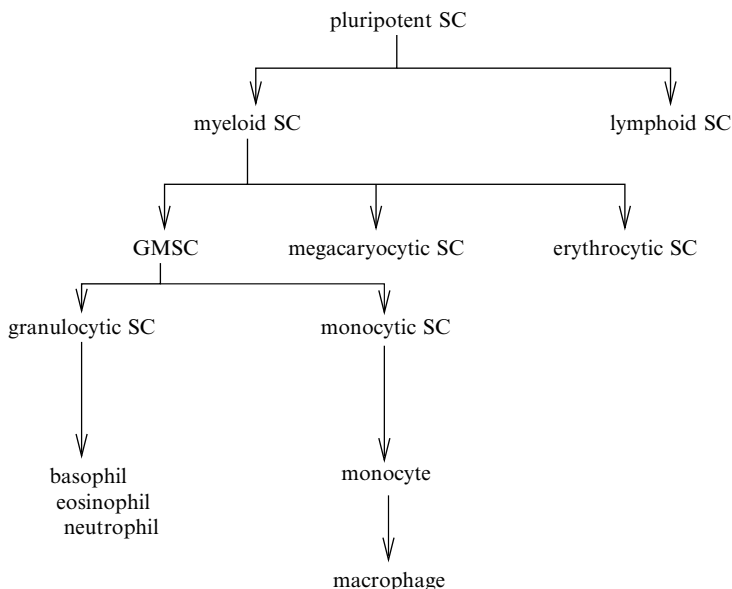


Fig. 2.1 Hematopoiesis with a focus on granulocytic and monocytic lineages (SC: stem cell, GMSC: granulocyte–monophage stem cell). After birth, hematopoiesis occurs in the bone marrow. Under the influence of stimulators, multipotent stem cells of the bone marrow differentiate into progenitors (committed stem cells). The first differentiation leads to myeloid and lymphoid stem cells. The progenitors undergo several divisions to give rise to stem cells with limited differentiation potential, the specific progenitors and precursors of a single blood cell lineage. Lymphoid stem cells (progenitors) produce B and T lymphocytes. Myeloid stem cells, or colony-forming unit **CFUgemm** (granulocyte–erythroid–macrophage–megakaryocyte) give birth to progenitors of many lineages (granulocytic, monocytic, erythrocytic, and megakaryocytic). Generator CFUgemm leads to more differentiated progenitors: (1) **CFUgm** (granulocyte–macrophage) that give rise to **CFUg** (granulocyte) and **CFUm** (macrophage); (2) **CFUeo** (eosinophil); (3) **CFUb** (basophil); (4) **CFUe** (erythroid); and (5) **CFUmeg** (megakaryocyte). Precursors have lost self-renewal capacity. They include myeloblasts, monoblasts, lymphoblasts, proerythroblasts, and megakaryoblasts. Several shared modifications characterize cell maturation: reduction in cell size and nucleocytoplasmic ratio and chromatin condensation. Precursors lead to mature blood cells after 3 to 5 divisions according to the cell lineage (one precursor can give birth to up to 32 daughter cells). Mature blood cells enter blood. Lymphocytes and monocytes can differentiate in tissues after their blood journey. Hematopoiesis is composed of several compartments: (1) multipotent stem cells such as colony-forming unit-spleen (**CFUs**), (2) progenitors, (3) precursors, and (4) mature cells (granulocytes, monocytes, lymphocytes, erythrocytes, and thrombocytes).

The turnover of hematopoietic cells¹ is estimated to be about 10^{12} cells per day [39]. All blood cell types are produced in the bone marrow from *pluripotent stem cells* (PSC; Fig. 2.1). Hematopoietic stem cells and derived progenitor cells yield blood resupply during the entire life. Pluripotent stem cells give birth via

¹ αιμoατικός: associated with blood, ποτεo: to do.

multi- and unipotent stem cells (USC) to the different lineages via proerythroblasts and erythroblasts, megakaryoblasts, myeloblasts and promyelocytes, and lymphoblasts.

2.1 Hematopoietic Stem Cells

Hematopoietic stem cells (HSC) generate a set of progenitors for erythroid, lymphoid, megakaryocytic, and myeloid lineages. Another type of multipotent cells resides in the bone marrow — *mesenchymal stem cells* — that can differentiate into osteoblasts, chondrocytes, and adipocytes. Most primitive hematopoietic stem cells are quiescent.

Two subpopulations of hematopoietic stem cells exist: (1) long-term quiescent (reserve) and (2) active hematopoietic stem cells [40]. In the bone marrow, endosteal regions composed of osteoblasts, osteoclasts, as well as vascular and CXCL12^{high} reticular cells, and central regions that lack osteoblasts form inhibitory and stimulatory (proliferative) zones, respectively. These 2 adjoining, but separate regions enable the coexistence of 2 different subpopulations (states) of hematopoietic stem cells.

The pool of hematopoietic stem cells comprises clonal subtypes with different lineage potential and self-renewal capacity. Aging affects the function and composition of mature blood cell compartments with a decline in lymphoid lineage potential and increased myeloid lineage commitment [41]. The gene program that specifies lymphoid fate is downregulated with age. Myeloid-biased hematopoietic stem cells produce high levels of signaling lymphocytic activation molecule family

Table 2.1. Members of the signaling lymphocytic activation molecule family (SLAMF) of immune receptors, or cluster of differentiation CD2 receptor family. They are synthesized in various subsets of immunocytes (T, B, and NK cells; BLAME: B-lymphocyte activator macrophage-expressed membrane protein; BLAST: B-lymphocyte activation surface marker; CD2F: CD2 family member; CD84H: CD84 homolog; CRACC: CD2-like receptor activating cytotoxic cells; CS: CD2 subset; Ly: lymphocyte surface antigen; NAIL: NK-cell activation-inducing ligand; NKR: NK-cell type-1 receptor; nLy: novel lymphocyte antigen; NTBA: natural killer-, T-, and B-cell antigen; SF: signaling lymphocytic activation molecule family member).

Member	Other aliases
SLAMF1	CD150, SLAM
SLAMF2	CD48, BLAST1
SLAMF3	CD229, Ly9
SLAMF4	CD244, NAIL, NKR2B4
SLAMF5	CD84, Ly9β
SLAMF6	CD352, Ly108, NTBA, SF2000
SLAMF7	CD319, nLy9, CRACC, CS1
SLAMF8	CD353, BLAME
SLAMF9	CD2F10, CD84H1, SF2001

member SLAMF1 (SLAMF1^{high} HSCs), or CD150 (Table 2.1), possess a stronger self-renewal potential, and predominate the stem cell pool in the aging body, whereas those that generate lower levels of SLAMF1 (SLAMF1^{low} HSCs) have a balanced lineage output.

Hematopoiesis depends on hematopoietic stem cell survival, self-renewal, and differentiation, hence mainly on the balance between quiescence (reserve) and cell division (regeneration of its pool and blood tissue). Lifetime hematopoiesis indeed relies on the capacity of hematopoietic stem cells to self-renew and to generate blood cells according to the body's needs. Blood homeostasis, hence hematopoiesis, requires long-term retention of hematopoietic stem cells in quiescence to maintain hematopoietic regenerative capacity. In the absence of a need of strong blood regeneration, the bone marrow, which is the site of blood cell formation from hematopoietic stem cells, maintains the circulating cell pool because mature blood cells continuously undergo senescence with a given degradation rate.

Tissue regeneration depends, at least partly, on stem cells, hence on their metabolic regulation. Hematopoietic stem cells are highly sensitive to energetic and oxidative stress; they shift between quiescence and proliferation according to the context. Kinase STK11, or LKB1, and its substrate AMPK coordinate metabolism with cell fate, thereby adapting cellular energetics with stem cell maintenance or tissue regeneration. The primary function of LKB1 in adult tissues is its inhibition of cell division, hence preventing tissue overgrowth. Enzyme LKB1 also controls cell survival and proliferation, as well as mitochondrial function and energy homeostasis in hematopoietic stem cells [42]. Inactivation of and deficiency in LKB1 in adult mice causes loss of HSC quiescence and subsequent depletion of all hematopoietic derived cells, as well as mitochondrial defects, alterations in lipid and nucleotide metabolism, and depletion of cellular ATP [42–44]. Hematopoietic stem cells relies more strongly on LKB1 for cell cycle regulation and survival than committed hematopoietic progenitor and precursor cells [42, 44]. However, LKB1 is needed for HSC maintenance via AMPK-dependent and -independent mechanisms [42, 43]. Metabolic sensor LKB1 indeed controls chromosome stability in HSCs via an AMPK-independent process. The metabolic control in hematopoietic stem cells yields an additional metabolic checkpoint of the cell division cycle. The bone marrow niche controls by sending proper cues the activity of hematopoietic stem cells during regenerative hematopoiesis.

The transcriptional regulation of cell quiescence relies on cell cycle regulators (e.g., P53, retinoblastoma protein, cyclin-D, and cyclin-dependent kinase inhibitors CKI1a and CKI1b) and factors with specific functions in hematopoietic stem cells (e.g., early growth response factor EGR1, forkhead box protein FoxO, DNA sequence GATA-binding protein GATA2, growth factor-independent transcription repressor GFI1, and Runx1).² The transcription factor, nuclear receptor-related

²Cell quiescence is associated with: (1) downregulation of cyclin-D1; (2) upregulation of cyclin-dependent kinase inhibitors CKI1a and CKI2a; (3) action of T-cell-intrinsic quiescence factor FoxO1; and (4) activation of P38MAPK [45]. Factor FoxO1 maintains cell quiescence and impedes T-cell activation by self-antigens, but does not prevent polyclonal T-cell activation. Polyclonal

protein NuRR1, or NR4a2, prevents the entry into the cell cycle [46]. Factor NR4a2 may upregulate cyclin-dependent kinase inhibitor CKI2c.

Connexin-43, a constituent of gap junctions, is synthesized in hematopoietic stem and progenitor cells. However, it is downregulated during the differentiation of hematopoietic stem cells to progenitors. Connexin-43 regulates the content of reactive oxygen species inside stressed hematopoietic stem cells, as it enables ROS transfer to supporting stromal cells in the hematopoietic environment [45]. Whereas low ROS levels are needed for cell activity (Vol. 4 – Chap. 10. Other Major Signaling Mediators), a sustained increase in ROS production during stress (and aging) can prevent self-renewal and cause senescence. Hyporegenerative capacity and cell cycle arrest can result from high levels of ROS and activation of P38MAPK and FoxO1 agents.

The number of hematopoietic stem cells is assessed to be of the order of 10000 cells. However, the blood cell production rate varies with mammal size. In addition, the larger the species size, the faster the proliferation rate. The size of the hematopoietic stem cell pool can be associated with allometric scaling, which is defined by a simple power law between a given observable y and the mass m of the organism:³

$$y = y_0 m^p. \quad (2.1)$$

The number of active stem cells in adult mammals evaluated by measuring the number of circulating reticulocytes scales with body mass with exponent 3/4 [47].

2.1.1 LSK Hematopoietic Stem Cells

Hematopoietic stem cells belong to a bone marrow cell population that expresses high levels of stem cell antigen-1 (SCA1) and plasmalemmal stem cell factor receptor (SCFR),⁴ but not plasmalemmal markers of lineage-committed hematopoietic cells (Lin-).⁵ This stem cell subset is hence defined as Lin-, SCA1+, SCFR (KIT)+ (LSK) cells.

T cells diversify their activities, unlike antigen-specific T-cell clones, but generally remain restricted to certain antigens. On the other hand, polyclonal immunoglobulins (antibodies) are combination of immunoglobulins secreted against a specific antigen, each identifying a different antigen epitope (antigenic determinant) and generally obtained from different B-cell clones.

³Allometric scaling can also be applied to basal metabolic rate, heart rate, arterial radii, etc. Most of the scaling powers $-p$ are multiples of 1/4. Computed power 3/4 can be related to a surface-to-volume ratio.

⁴Membrane-bound stem cell factor, or cellular kinase in tyrosine (KIT or cKIT) ligand, after proteolytic cleavage, binds and activates SCFR receptor. Membrane-bound stem cell factor stimulates adhesion of hematopoietic stem and progenitor cells to stromal cells, because it activates integrins.

⁵A lineage marker negative phenotype (Lin-) corresponds, in mice, to CD2-, CD3-, CD4-, CD5-, CD8-, CD161c-, PTPRc-, Ly76-, Ly6g-. Hematopoietic stem cells can be precisely distinguished from other hematopoietic progenitors by the plasmalemmal expression of signaling lymphocytic activation molecules (SLAM), such as SLAMF1, SLAMF2, and SLAMF4 [48].

2.1.2 Hemangioblast

Both hematopoietic and endothelial cells arise from a mesoderm-derived VEGFR2+ common precursor, the so-called hemangioblast.⁶ Hematopoietic progenitor cells arise from these bipotential precursors via a subset of early endothelial cells, i.e., differentiated endothelial cells that have a hematopoietic potential and form the *hemogenic endothelium* [49].

TIE2^{high}, SCFR+ hemogenic endothelium is transiently generated. Hemangioblasts isolated from differentiated mouse embryonic stem cells in culture generate tightly adherent structures (stage 1) and then non-adherent round cells that proliferate to generate a mature blast colony (stage 2) [50].⁷

During embryogenesis, SCA1+, SCFR+ (CD117+), CD41+ (Itg α_{2B} +) hematopoietic stem cells emerge directly from the aortic floor into the dorsal aortic lumen, at least in some species, following egress of Runx1+ endothelial cells from the aortic ventral wall into the subaortic space [51, 52]. Cadherin-5+ (or vascular endothelial [VE]-cadherin) endothelial precursors or hemogenic endothelial cells transiently possess the ability to give rise to multipotent hematopoietic stem and progenitor cells during vertebrate development (*endothelial-hematopoietic transition*) [53]. These cells afterward enter the blood circulation to colonize and differentiate in hematopoietic organs.

2.2 Biological Models of Hematopoiesis

Hematopoiesis relies on classical and alternative pathways. Long-term hematopoietic stem cells give rise to short-term hematopoietic stem cells that have a transient ability to self-renew and differentiate into multipotent progenitors [54].

The latter lack the capacity of self-renewal, but retain multipotency, creating a series of intermediate progenitors, such as common lymphoid and myeloid progenitors that further differentiate into lymphoid and granulocyte-macrophage progenitors, respectively.

Lymphoid-primed multipotent progenitors can develop lymphoid and myeloid progeny, but not erythroid and megakaryocytic cells.

⁶Marker VEGFR2 is also called fetal liver kinase FLK1 and kinase insert domain receptor (KDR). VEGFR2+, cadherin-1+ precursors can differentiate into endothelial, primitive and definitive hematopoietic progenitor cells, cardiomyocytes, and mural cells.

⁷At stage 1, a majority of cells is positive for the endothelial marker, angiopoietin-1 TIE2 receptor (a.k.a. Tyr endothelial kinase [TEK]), whereas a minority of cells is positive for α_{2B} integrin (or CD41) that defines hematopoietic engagement. Afterward, the percentage of CD41+ cells rises and most cells are rapidly CD41+, TIE2- cells. Transient TIE2^{high}, SCFR+ cell population that contains both CD41- and CD41+ cells hence represents a transitional population from which definitive hematopoietic progenitors originate.

Granulocyte–macrophage progenitors generate myelomonocytic and megakaryocytic–erythrocyte progenitors. However, the separation between the myeloid and megakaryocytic–erythroid cell lineages can occur before the development of common myeloid progenitors.

Myelo-erythroid progenitors include pregranulocyte–macrophage precursors that give rise to granulocyte–macrophage progenitors, whereas prepremegakaryocytic–erythroid precursors yield preCFUe (CFU erythroid), CFUe, and megakaryocytic–platelet (MKP) compartments.

2.2.1 *Arborescence Models of Hematopoiesis*

An arborescence model of hematopoiesis assumes branches for the differentiation of blood cells [55]. This model states that: (1) megakaryocytes as well as erythroid and myeloid cells from a *multipotent progenitor* (MPP), *lymphoid-primed multipotent progenitor* (LMPP), *early lymphoid progenitor* (ELP), or *common myeloid progenitor* (CMP); and (2) natural killer cells and B and T lymphocytes from a *common lymphoid progenitor* (CLP).

Another tree-like model supposes that: (1) a *common myeloid–lymphoid progenitor* (CMLP) gives rise to (1.1) a *myeloid–T-cell progenitor* (MTP) that differentiates into myeloid and T lymphocytes and (1.2) *myeloid–B-cell progenitor* (MBP) that gives rise to myeloid and B lymphocytes; and (2) a *common myeloid progenitor* generates myeloid cells and a *myeloid–erythroid progenitor* (MEP), a source of erythroid cells and megakaryocytes.

A third model proposes that erythroid potential is lost early from a *common myeloid progenitor* that differentiates into *granulocyte–monocyte progenitor* (GMP) with myeloid potential and *myeloid–erythroid progenitor*, whereas a *common lymphoid progenitor* leads to lymphoid cells.

Arborescence Model Drawbacks

Lymphoid progenitors can have a myeloid potential and differentiate into myeloid cells (macrophages and dendritic cells). Therefore, models of early segregation of lymphoid and myeloid differentiation axes are not validated by observations.

2.2.2 *Lymphoid Progenitors*

Most Lin[−], SCA1⁺, SCFR⁺, FLT3⁺ lymphoid-primed multipotent progenitors lack erythroid and megakaryocytic potential. Several lymphoid progenitors that are able to generate B and T lymphocytes and natural killer cells include: (1) Lin[−], SCA1⁺, SCFR⁺, FLT3^{high}, VCAM1[−] lymphoid-primed multipotent progenitors;

(2) SCFR^{high} early lymphoid progenitors that express recombination-activating gene RAG1;⁸ and (3) SCFR[−], PTPRc⁺, CLP2 (type-2 common lymphoid progenitor) progenitors that express the gene encoding the preT-cell antigen receptor.⁹

Early progenitors with lymphoid and myeloid potential (EPLM), *lymphoid-primed multipotent progenitors* (LMPP), and *thymic progenitors* that have both lymphoid and myeloid potential receive many signals that govern their fate. This destiny depends on the type of growth factors as well as strength and duration of signaling.

Lymphoid-primed multipotent progenitors have both lymphoid and myeloid potential, with high granulocytic, monocytic, and lymphoid potential, but low potential for megakaryocyte or erythroid development [55]. However, an LMPP subpopulation that expresses the thrombopoietin receptor has a megakaryocytic and erythroid potential.

The earliest thymic progenitors retain myeloid potential, hence can contribute to thymic granulocyte and macrophage populations, but not B cells. Early T-cell progenitors, or CD4[−], CD8[−] *double-negative cells* (DN), comprise:

1. SCFR^{high10} early thymocyte progenitors (ETP);
2. SCFR^{high}, CD44⁺, CD25[−] DN1 cells;
3. SCFR^{high}, CD44⁺, CD25⁺ DN2 cells;
4. SCFR[±], CD44[−], CD25⁺ DN3 cells; and
5. SCFR[−], CD44[−], CD25[−] DN4 cells.

Notch-1 is required for T-cell development. When it is lacking in the thymus, thymocyte progenitors bifurcate toward B-cell development.

Early thymocyte progenitors (ETP) can be subdivided according to expression of stem cell protein Tyr kinase-1 (STK1) receptor¹¹ and chemokine CCR9 receptor. Only STK1⁺ or CCR9⁺ *thymus-settling progenitors* have B-cell potential [55]. Double-negative DN1 and DN2 cells are not committed to T-cell development, but give rise to NK and myeloid cells.

The pairwise relationship model of hematopoiesis [55] does not take into account branching patterns that determine preferred route for a given cell fate. It displays in a circle blood cell sectors (megakaryocyte, erythrocyte, basophil or mastocyte, eosinophil, neutrophil, monocyte, dendritic cell,¹² and B, NK, and T cell) and arcs

⁸The recombination-activating gene, or RING finger gene Rnf74, encodes enzymes that act in the rearrangement of immunoglobulin genes and T-cell receptors during VDJ recombination.

⁹Two subpopulations of common lymphoid progenitors — CLP1 and CLP2 — coexist in the bone marrow and generate lymphocytes. Type-1 common lymphoid progenitors (CLP1 cells) are IL2Rα⁺ (CD25), PTPRc[−], SCFR[−] cells, whereas CLP2 cells are IL2Rα⁺, PTPRc⁺, SCFR[−] cells.

¹⁰I.e., CD117^{high}.

¹¹A.k.a. CD135, fetal liver kinase FLK2, and FMS-like Tyr kinase receptor FLT3.

¹²Dendritic cells can have both lymphoid and myeloid origins, but have a developmental program that is independent from that of lymphoid and myeloid cells. Cells that can differentiate into dendritic cells often also have the potential to become B, T, and NK cells.

inside the disk assigned for each progenitor and precursor with overlaps (as a final cell fate can be reached via several types of intermediate progenitors) or involved transcription factors in hematopoietic stem cells.

2.3 Stem Cell Niches

The embryonic origin of adult hematopoietic stem cells (multipotent progenitors) is the aorta–gonad–mesonephros region. Hematopoietic stem cells subsequently colonize fetal and adult hematopoietic organs, i.e., fetal liver and spleen on the one hand and, in adults, the bone marrow as well as spleen and liver during hematopoietic stress on the other. Colony-forming units in the spleen reconstitute transiently multipotent progenitors.

2.3.1 *Types of Bone-Marrow Niches*

Different niche types for hematopoietic stem cells exist: (1) osteoblastic and (2) vascular bone marrow HSC niches. In adults, hematopoietic stem cells have their own bone marrow microenvironment,¹³ close to the endosteal¹⁴ surface of bone marrow cavities in trabecular regions of long bones, whereas more differentiated hematopoietic progenitors are mainly located in the central bone marrow region [56]. The endosteal niche is characterized by a sinusoidal endothelium. Changes in the HSC niche do not allow appropriate HSC maintenance in vivo.

In addition to osteoblastic niches, the vascular bone marrow HSC niche demarcated by bone marrow sinusoidal endothelial cells is a second specialized HSC microenvironment in the bone marrow, with a large quantity of SLAMF1+ hematopoietic stem cells attached to the fenestrated endothelium of bone marrow sinusoids. Endothelial cells of bone marrow sinusoids yield a cellular platform for the differentiation of lineage-committed progenitors such as megakaryocytic progenitor cells (CFU megakaryocyte).

Bone marrow vascular and endosteal niches cooperate to control HSC quiescence and self-renewal. A small number of hematopoietic stem cells is constantly released into the blood circulation. Endothelial cells of bone marrow sinusoids differ from endothelial cells of the microvasculature of any other organ. These endothelial cells

¹³Extramedullary transient hematopoiesis, in the liver or spleen, occurs after bone marrow stress; but after repair, bone marrow hematopoiesis follows.

¹⁴The endosteum is a bone remodeling site characterized by high concentrations of calcium ions. Extracellular Ca^{2+} concentration is assessed by calcium-sensing receptors.

express cytokines such as chemokine CXCL12,¹⁵ like osteoblasts, and adhesion molecules for HSC mobilization, homing,¹⁶ and lodging in endosteal bone marrow HSC niches [56]. SLAMF1+ hematopoietic stem cells close to sinusoids may monitor the concentration of blood elements.

Hematopoietic stem cell niches regulate the stem cell maintenance in cooperation with endosteal cells (osteoblasts and osteoclasts) at the interface between bone and bone marrow, as well as vascular and perivascular cells around bone marrow sinusoids. The specific microenvironment promotes stem cell survival and self-renewal and regulates cell differentiation and migration according to the body's need. Endosteal, vascular, and perivascular cells cooperate and contribute to the HSC maintenance, expansion, and location. Homeostasis of hematopoietic stem cells requires many factors (Table 2.2) [57].

Many plasmalemmal receptors control the location of hematopoietic stem cells, among which the calcium-sensing receptor [61].¹⁷ Calcium-sensing receptors are particularly expressed by LSK cells.

2.3.2 *Cells of Bone Marrow Niches*

Osteopontin+, N-cadherin+ osteoblasts that line the endosteal surface of trabecular bone, bone marrow sinusoid endothelial cells, CXCL12-secreting stromal cells, adipocytes, and macrophages constitute functional niches of hematopoietic stem cells in adult bone marrow.

The adipocyte number is inversely correlated with the hematopoietic activity in the niche. Density of hematopoietic stem cells and progenitors decays in the adipocyte-rich bone marrow. Bone marrow adipocytes operate as inhibitors of hematopoiesis [59].

On the other hand, in vitro, undifferentiated stromal cells and preadipocytes support hematopoiesis, as they produce growth factors, such as granulocyte-macrophage (CSF2) and granulocyte (CSF3) colony-stimulating factor. Moreover,

¹⁵Chemokine CXCL12 can induce motility, chemotaxis, and adhesion of cells expressing its receptor, CXC-chemokine receptor-4, as well as secretion of matrix metalloproteinases and angiogenic factors, such as vascular endothelial growth factor. Association of CXCL12 and CXCR4 is required for retention and maintenance of adult hematopoietic stem cells.

¹⁶Homing corresponds to recruitment of circulating hematopoietic stem cells to the bone marrow vasculature and subsequent extravasation that requires adhesion molecules, such as selectins and integrins. Small GTPases Rac1 and Rac2 are also implicated in homing and retention of hematopoietic stem cells in the endosteal bone marrow HSC niches.

¹⁷The endosteal niche for hematopoietic stem cells is characterized by a high Ca^{2+} concentration (more than 20-fold the plasmatic one), due to active bone remodeling in the endosteum by osteoclasts.

Table 2.2. Factors of the maintenance of hematopoietic stem cells (Sources: [57, 58]; MPL: myeloproliferative leukemia virus proto-oncogene product). Stromal and parenchymal cells of the bone marrow (mesenchymal progenitors, osteoblasts, fibroblasts, adipocytes, and reticular and endothelial cells), endosteal cells (undifferentiated bone-lining cells, bone-forming osteoblasts, and bone-resorbing osteoclasts), and vascular and perivascular cells of bone marrow sinusoids secrete factors that promote HSC maintenance. Osteoblasts secrete angiopoietin, thrombopoietin, and CXC-chemokine ligand-12 (CXCL12). Angiopoietin (also secreted by megakaryocytes and perivascular cells in the bone marrow) and thrombopoietin (supplied by blood or produced by stromal cells) promote HSC quiescence; CXCL12 (also secreted by reticular and perivascular cells) regulates HSC migration (CXCR4: CXC-chemokine receptor-4). Alternative splicing creates many epican variants (a.k.a. CD44 and homing cell adhesion molecule [HCAM]), especially the smallest epican_S (or hematopoietic CD44h) that is strongly expressed on hematopoietic cells and long epican_L (CD44v). Due to diversity of extracellular domain, epican interacts with numerous ligands (hyaluronate, fibronectin, collagen-1 and -4, serglycin, osteopontin, etc.).

Factor	Receptor, partner
Angiopoietin	TIE1/2
N-cadherin	Ca ²⁺ -dependent N-cadherin receptor
Calcium	Ca ²⁺ -sensing receptor
CXCL12	CXCR4
Osteopontin	Epican (CD44), $\alpha_v\beta_3$ -integrin
Prostaglandin-E2	Cyclooxygenase and synthase
Sonic Hedgehog	Patched
Stem cell factor	Stem cell factor receptor (KIT)
Thrombopoietin	Thrombopoietin receptor (MPL)

adipocytes secrete neuropillin-1, lipocalin-2,¹⁸ adiponectin, and tumor-necrosis factor- α that can preclude hematopoietic cell proliferation. Although adipocytes prevent expansion of hematopoietic progenitors, they preserve hematopoietic stem cell pool.

2.3.3 Crosstalk between Niche-Resident Cells

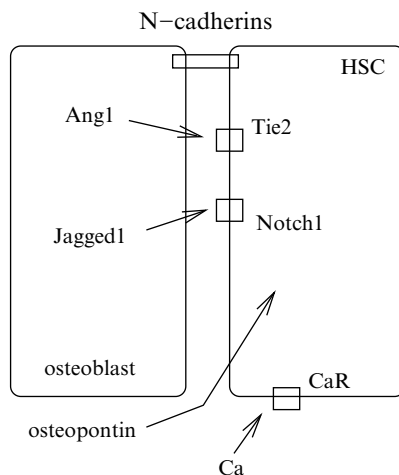
Molecular crosstalk between hematopoietic stem cells and other cellular constituents of endosteal niches regulate the balance between HSC self-renewal and differentiation. Cellular adhesions between stem cells and stromal cells and/or the extracellular matrix anchor stem cells within the niche and regulate stem cell behavior. Whereas β_1 - and β_7 -integrins are not necessary to maintain retention of adult hematopoietic precursors in the bone marrow, other agents, such as membrane-

¹⁸A.k.a. neutrophil gelatinase-associated lipocalin (NGAL) and migration-stimulating factor inhibitor (MSFI).

Fig. 2.2 Endosteal niche of hematopoietic stem cells.

Interactions between hematopoietic stem cells and osteoblasts

(Sources: [56, 60]).



bound stem cell factor¹⁹ and its receptor SCFR, as well as CXCR4 can mediate their sequestration in the niche [62].

Osteoblasts secrete multiple cytokines that promote the proliferation of hematopoietic cells. Specialized spindle-shaped N-cadherin-expressing osteoblasts of the endosteum directly contact hematopoietic cells via N-cadherin.²⁰ Many stromal cell lines of endosteal bone surfaces that are involved in bone modeling, are required in HSC maintenance. The endosteal niche can contain quiescent and self-renewing hematopoietic stem cells.

Osteopontin that prevents the proliferation of hematopoietic stem cells may maintain HSC quiescence (Fig. 2.2). *Angiopoietin-1* at the osteoblast surface interacts with its receptor Tyr kinase with Ig and EGF homology domains TIE2 on stem cells to maintain stem cell quiescence in the niche.

Certain situations such as inflammation trigger the activity of osteoclasts along the stem cell niche, secretion of enzymes (cathepsin-G, elastase, MMP9, etc.) and cytokines (interleukin-8), and mobilization of progenitors from the bone marrow to the circulation [63]. Osteoclasts require TNFSF11 from osteoblasts for proliferation and bone resorption. Activating osteoclasts via either TNFSF11 or other stimuli leads to emigration of hematopoietic stem cells into the circulation. On the other hand, increased osteoblast activity via parathyroid hormone receptor or inactivation of the bone morphogenetic protein receptor-1A causes a proliferation of hematopoietic stem cells.

¹⁹A.k.a. hematopoietic growth factor and steel factor (SLF).

²⁰Receptor Tyr kinases TIE1 and TIE2 are required in HSC maintenance in HSC microenvironment. Receptor TIE2 activated by angiopoietin-1 secreted by osteoblasts upregulates N-cadherin expression in hematopoietic stem cells and maintains HSC quiescence via CKI1 a cyclin-dependent kinase inhibitor.

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