22.2 Haematopoiesis 5172 22.2.1 Cellular and molec

22.2 Haematopoiesis 5172 22.2.1 Cellular and molecular basis of haematopoiesis 5172 Paresh Vyas and N. Asger Jakobsen

CONTENTS 22.2.1 Cellular and molecular basis of haematopoiesis 5172 Paresh Vyas and N. Asger Jakobsen 22.2.2 Diagnostic techniques in the assessment of haematological malignancies 5181 Wendy N. Erber 22.2.1 Cellular and molecular basis of haematopoiesis Paresh Vyas and N. Asger Jakobsen ESSENTIALS Haematopoiesis involves a regulated set of developmental stages by which haematopoietic stem cells (HSCs) produce haematopoietic progenitor cells which in turn differentiate into more mature haem- atopoietic lineages. These then provide all the key functions of the haematopoietic system. Development Haematopoiesis occurs in distinct waves during development. Definitive HSCs first develop within the embryo in specialized re- gions of the dorsal aorta and umbilical arteries and then seed the fetal liver and bone marrow. HSC characteristics differ based on their site of development and age of the organism. Haematopoietic stem cells At the single-cell level, these have the ability to reconstitute and maintain a functional haematopoietic system over extended periods of time in vivo. They (1) have a self-renewing capacity during the life of an organism, or even after transplantation; (2) are multipotent, with the ability to make all types of blood cells; and (3) are relatively qui- escent, with the ability to serve as a deep reserve of cells to replenish short-lived, rapidly proliferating progenitors. In vivo transplantation models are currently the only reliable assays of HSC activity. Haematopoietic progenitor cells These are unable to maintain long-term haematopoiesis in vivo due to limited or absent capacity for self-renewal.

Their rapid pro- liferation and cytokine responsiveness enables increased blood cell production under conditions of stress. Lineage commitment means limited cell type production. The haematopoietic stem cell niche An anatomically and functionally defined regulatory environment for stem cells modulates self-renewal, differentiation, and prolif- erative activity of stem cells, thereby regulating stem cell number. Niche function is important in maintaining haematopoietic integrity and niche dysfunction may contribute to haematopoietic disease. Niches for HSCs are dynamic, changing during development and with physiological stress. HSCs naturally traffic into and out of the niche, a feature that can be exploited for stem cell transplantation or harvesting, respectively. Bone marrow transplantation Haematopoietic reconstitution during bone marrow transplant- ation is mediated by a succession of cells at various stages of development. More mature cells contribute to repopulation im- mediately following transplantation. With time, cells at progres- sively earlier stages of development are involved, with the final stable repopulation being provided by long-lived, multipotent HSCs. Long-term haematopoiesis is sustained by a relatively small number of HSCs. Haematopoiesis through development and into adult life Adult humans produce approximately 300 to 1000 billion blood cells per day (Table 22.2.1.1). The vast majority of these are myeloid cells—platelets, erythroid cells, and granulocytes. Haematopoiesis is the process by which blood cells are made throughout develop- ment (embryonic life) and adult life by transiting through a hier- archy of haematopoietic stem and progenitor cells (HSPCs). This chapter will summarize the cellular and molecular basis of haem- atopoiesis as a prelude to a deeper understanding of benign and malignant blood diseases. 22.2 Haematopoiesis

22.2.1 Cellular and molecular basis of haematopoiesis 5173 Primitive haematopoiesis Haematopoiesis occurs in waves and at multiple discrete anatomical sites that change through development (Fig. 22.2.1.1). In humans, like other vertebrates, the initial wave of haemato- poiesis occurs in the extraembryonic yolk sac blood islands from weeks 3 to 6 of gestation (Fig. 22.2.1.2). The yolk sac primarily pro- duces primitive erythroid cells (termed primitive erythropoiesis) expressing embryonic globins that deliver oxygen to tissues in the rapidly growing embryo. Primitive haematopoiesis also produces myeloid and lymphoid cells (macrophages and natural killer cells). Interestingly, the developmental potential of embryonic haem- atopoiesis closely resembles haematopoietic cells derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). Embryonic definitive haematopoiesis Primitive haematopoiesis is transient and is replaced by definitive haematopoiesis that sustains blood production throughout devel- opment and postnatal life. Embryonic definitive haematopoietic ac- tivity is detected at 4 to 5 weeks of gestation, in a region around the ventral wall of the dorsal aorta (Fig. 22.2.1.3). In early development, blood cells arise in close connection with the vascular structures (both in the yolk sac and the dorsal aorta) giving rise to the notion that there may either be a common precursor cell population that gives rise to both blood and blood vessel cells called a haemangioblast, or that haematopoiesis arises directly from specialized ‘haemogenic’ endothelial cells such as those lining the ventral aspect of the dorsal aorta. In mice and other animals, studies have shown that defini- tive haematopoietic stem cells (HSCs) with serially transplantable activity and long-term engraftment capacity are found in the dorsal aorta. It is still a matter of debate whether HSCs arise from the em- bryo proper (the dorsal aorta) or by colonization from the yolk sac. A large transient pool of HSCs has been identified in the placenta of mice around the time of aorta–gonad–mesonephros HSC develop- ment. It remains to be determined whether an equivalent population of HSCs exists in the developing human placenta. Fetal liver haematopoiesis HSCs are then detected in the developing fetal liver, spleen, and thymus from 6 to 22 weeks in humans,

where they expand and dif- ferentiate into committed progenitor cells. Recent evidence sug- gests that the critical niche supporting HSCs is the fetal vasculature. Expansion and differentiation of HSCs allows for development of definitive red cells, myeloid cells, and lymphoid cells (T cells that YOLK SAC AGM Weeks 2.5–4 of gestation Weeks 4–10 of gestation Week 5 of gestation until term Mainly postnatal LIVER SPLEEN BONE MARROW Fig. 22.2.1.1 Changing anatomical locations of haematopoiesis through development. Haematopoiesis is initially detected in the extraembryonic yolk sac, then in the embryo, in the aorta–gonad–mesonephros (AGM), the adjacent umbilical arteries and vitelline vessels, and the placenta. It then shifts to the fetal liver and finally to the bone marrow. Table 22.2.1.1 Cellular components of blood Red cells White cells Platelets Number/litre of blood 4–6 × 1012 4–11 × 109 c.60% are myeloid cells 150–400 × 109 Total cell number (blood volume 5 litres) 20–30 × 1012 20–55 × 109 750–2000 × 109 Cell lifespan 120 days Myeloid cells: 1–5 days Lymphoid cells: weeks to years 2–4 days Daily myeloid cell production 1.7–2.5 × 1011 12–30 × 109 3.5–10 × 1011

 develop in the thymus and B cells in the marrow). It is unclear whether HSCs from the dorsal aorta migrate to colonize fetal liver and other embryonic sites or whether they arise de novo at these other sites. Adult bone marrow haematopoiesis HSPCs seed the bone marrow (BM) during fetal life but only make a small proportion of blood until late in gestation. It is not clear whether HSCs must first reside in the fetal liver before seeding the BM. However, at the time of birth, haematopoiesis switches from fetal liver to BM as the liver is repurposed to serve its roles in adult life. The mechanisms resulting in this remarkable switch in anatom- ical location are unclear, but it is very likely that the changing blood supply to the liver plays a critical role, wherein oxygenated blood from the umbilical cord is replaced by relatively deoxygenated blood from the portal vein that is rich in metabolites ingested from the gut. The marrow then sustains lifelong blood production, where HSPCs reside in both endosteal and vascular niches of the marrow cavity. In early life, haematopoiesis occurs in the diaphysis and epiphysis of the axial skeleton and the long bones. With age, the marrow cavity becomes fatty and haematopoiesis is progressively restricted to the axial skeleton. Given that HSPCs isolated from different developmental time points and anatomical locations (e.g. fetal liver, placental cord blood, BM, hESCs, and iPSCs) have been shown to have different func- tional potentials, this may have implications regarding the choice of stem cell sources for human transplantation therapies. Haematopoiesis: a hierarchical differentiation cascade HSPC populations give rise to all haematopoietic cells through a cascade of differentiation. Stem and progenitor are both highly heterogeneous populations. They are defined by two key proper- ties: variable ability to self-renew and a variable potential to differ- entiate into one or more mature blood cell types. Residing at the top of this hierarchy are HSCs. They likely number tens to a few hun- dreds of thousands of cells in adult life, giving rise to hundreds of millions of hierarchically organized, highly heterogeneous progenitor cells, which in turn differentiate into precursor cells that eventually mature into effector cells (Fig. 22.2.1.4). The current view of the haematopoietic cellular hierarchy will change over the coming years as we functionally and molecularly interrogate HSPCs at a single-cell level. Our understanding of the hierarchical relationships between populations, the plasticity of commitment, and the nature of the functional potential of popula- tions will increase as we better purify HSPC populations. Haematopoietic stem cells HSCs have extensive self-renewal capability and the potential to differentiate into all blood cell types. The remarkable ability of HSCs, at the single-cell level, to reconstitute and maintain a functional haem- atopoietic system over extended periods of time in vivo demonstrates these key properties. Self-renewal allows HSCs to be transplanted

between individuals, and the surviving HSCs engraft, proliferate, and differentiate for the life of the recipient. HSCs can be serially Fig. 22.2.1.3 Transverse section through the human fetal dorsal aorta at embryonic day 32 showing haematopoietic CD34+ cells clusters (arrowheads) associated with the ventral wall. CD34+ cells (haematopoietic and endothelial cells) are stained brown. Scale = 100 microns. From Tavian M, Hallais MF, Péault B (1999). Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. Development, 126, 793–803. (a) (b) Fig. 22.2.1.2 Yolk sac blood islands in a human fetus. (a) Transverse section of a three-somite human embryo (21 days) at the truncal level stained with anti-CD34 antibody. Paired dorsal aorta (da) ventrolateral to the neural tube (nt) and above the yolk sac (ys) and blood islands (bi). Scale = 200 microns. (b) Higher magnification of a solid haemangioblastic mesodermal cluster of CD34-expressing cells in a blood island of the yolk sac (brown). Scale = 25 microns. From Tavian M, Hallais MF, Péault B (1999). Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. Development, 126, 793–803.

22.2.1 Cellular and molecular basis of haematopoiesis 5175 transplanted for many generations between recipients. When HSCs are recruited into active haematopoiesis, they exit the G0 phase of the cell cycle, and their daughter cells may either be a replicate of the parent cells (self-renewal) or enter into a differentiation programme. This distinctive, asymmetric division process is the basis for long- term preservation of stem cells while enabling continued produc- tion of mature cells. The daughter cells that undergo differentiation proceed through a series of maturational cell divisions, culminating in the generation of progenitor cells. Most of our detailed understanding of HSCs has come from murine studies though some of the findings have also been con- firmed in humans. Contrary to previous assumptions, HSCs are a heterogeneous cell population through development and adult life. In the mouse, fetal HSCs show extensive prolifer- ation and tend towards greater lymphoid output. During adult life, there is a hierarchy of HSCs. The most primitive HSCs are rare, representing approximately 1 in 104 to 106 BM cells. These long-term stem cells are deeply quiescent and may replicate only once a year, which protects their genome from replicative mutational damage. These give rise to HSCs that cycle slightly more often. Adult HSCs are also heterogeneous with respect to their lineage output. Some are biased towards producing megakaryocyte–erythroid cells, others more myeloid cells, and finally yet others more lymphoid cells. Yet all HSCs, if required, can produce all lineages. With age, HSC numbers increase but they become less functional and those that are biased to pro- duce lymphocyte lineages diminish relative to myeloid-biased HSCs. This potentially contributes to the relative lymphoid de- ficiency and increase in myeloid diseases (myeloproliferative disorders, myelodysplasia, and acute myeloid leukaemia) seen in the elderly. Progenitor cells Progenitor cells are also hierarchically arranged. As they differen- tiate from stem cells and through the progenitor hierarchy they progressively lose self-renewal and become restricted in their dif- ferentiation potential such that multipotent progenitors give rise to oligopotent and finally monopotent progenitors. Progenitors are highly proliferative and very cytokine responsive. New studies of murine haematopoiesis suggest that, in contrast to the trans- plantation setting, the source of most blood cells produced daily during normal steady-state haematopoiesis is maintained by the continuing expansion of thousands of haematopoietic progenitor cells, each with a minimal contribution to mature progeny. Single- cell transplant studies in mice have also revealed a bypass pathway that produces long-term repopulating myeloid progenitors. This pathway may be operative under stress, as progenitor populations most readily respond to external stimuli in order to up- and down- modulate production of specific blood cell types. Progenitors differ- entiate into lineage-restricted precursor cells and

eventually mature effector cells of the haematopoietic system. These mature lineages include erythroid cells for oxygen transport, myeloid and lymphoid cells that provide immune defence, and megakaryocytes and plate- lets essential for haemostasis. HSC haemopoietic stem cells MPP multipotential progenitor/short-term HSC lymphoid primed multipotential progenitor myelolymphoid progenitor common myeloid progenitor granulocyte myeloid progenitor megakaryocyte erythroid progenitor megakaryocyte progenitor erythroid progenitor Quiescent Proliferate in response to stimuli Lymphopoiesis static cell numbers in steady state T-cell B-cells PC Dendritic Cells Natural Killer Cells Neutrophils Monocytes Monocyte DC Myeloid DC Mks/ Platelets Erythroid Cells Proliferation Self Renewal Quiescence HSC MPP LMPP CMP GMP MLP MEP MkP BFU-E Myelopoiesis 10 billion cells/day LMPP MLP CMP GMP MEP MkP BFU-E Fig. 22.2.1.4 One current model of human adult haematopoiesis. A self-renewing HSC gives rise to a multipotent progenitor (MPP) that gives rise to a lymphoid-primed MPP (LMPP), both of which may have short-term self-renewal capacity. The hierarchy following the LMPP further bifurcates into a multilymphoid progenitor (MLP) and a granulocyte macrophage precursor (GMP). The common myeloid progenitor (CMP) is likely to be a heterogeneous population that gives rise to the megakaryocyte/erythroid progenitor (MEP) and the GMP.

 Phenotypic characterization and isolation of HSPCs Attempts to purify stem cell populations have used a combination of approaches based on physical and biological properties and cell surface marker expression. Early work on murine BM revealed that transplantable HSCs co-purified with lymphocytes and led to the idea that HSCs are morphologically indistinguishable from lympho- cytes. Density gradient separation, such as Ficoll and Percoll gra- dients, are commonly used as a pre-enrichment step in stem cell purification protocols. Progenitor cells cycle actively, whereas HSCs are relatively quiescent. This difference has been exploited in techniques for HSC enrichment in mouse and human systems. For example, treatment of mice with the antimetabolite agent fluorouracil mark- edly reduces progenitor cells, while relatively sparing populations enriched in HSC activity. More recently, considerable progress has been made in prospect- ively isolating HSPCs using flow cytometry and cell surface markers. However, it is difficult to compare different immunophenotyping strategies with respect to quantifying the purity of the HSC popula- tion. Numerous factors, such as the source of HSC (umbilical cord vs marrow), route of transplant (intravenous vs intrafemoral), the type of immunodeficient mouse used for xenografting, and pretransplant manipulation of the cells can all affect the quantification of the purity of HSC populations. Figure 22.2.1.5 schematically illustrates how HSCs are isolated and tested for function. Similar approaches are used to isolate pro- genitor cells. Haematopoietic tissues are isolated, cells disassociated, and then labelled with panels of fluorescently conjugated antibodies. Cell populations can then be analysed and separated on a fluores- cence activated cell sorter. Early studies to isolate human HSCs found that approximately 1% of human BM cells express CD34. Isolation of CD34+ cells enriches for HSPCs and haematopoietic engraftment YS BM disassociate cells SSC FSC CD34 CD38 CD34 CD38 NOD–SCID mouse 1 2 long-term culture initiator cells (LTC-IC) cobblestone area forming assay (CAFC) colony assay liquid culture assay in vivo engraftment assay in vitro clonogenic assay CD34 CD38 FL AGM Fig. 22.2.1.5 Isolation of haematopoietic stem cells. HSCs can be isolated from different sources. Cells are initially disassociated and stained with multiple antibodies. They are then separated using a fluorescent activated cell sorter. Here mononuclear live cells are separated in gate 1. These live cells are then analysed for CD34 and CD38 expression. The live CD34+CD38− cell population is enriched for stem cell potential. Further purification can be undertaken on the

basis of additional cell surface markers such as CD45RA, CD90, and CD49f and efflux of dyes, for example, rhodamine. To test functionality of isolated (sorted) cells, cells can be tested in in vivo assays (transplanted into immunodeficient mice) and in vitro in long-term culture (long-term culture-initiating cell culture assay and cobblestone-area forming assay), clonogenic colony assays, and liquid culture assays.

22.2.1 Cellular and molecular basis of haematopoiesis 5177 when transplanted into irradiated nonhuman primates. Similarly, human CD34+ selected cells contain HSPCs capable of fully recon- stituting the haematopoietic system in humans after myeloablative chemotherapy and radiation therapy. Although a CD34-expressing population contains long-term repopulating HSCs, there is some evidence of an upstream deeply quiescent CD34− population that gives rise to the CD34+ HSCs. Approximately 5 to 25% of CD34+ cells also express low to moderate levels of CD90. CD90 expression by human haematopoietic cells decreases with differentiation, and most lineage-restricted progenitors are CD34+CD90−/low cells. Additional studies demonstrate that human HSCs do not express mature cell lineage markers (Lin−), CD45RA or CD38. Isolation of Lin− CD34+CD38−CD45RA−CD90+ cells provides a relatively easy method to sort for putative HSCs. Sorting for the integrin CD49f further enriches for HSCs. Others have combined some of these markers with the ability of HSC to efflux dyes (e.g. the mito- chondrial dye rhodamine-123). HSCs, but not progenitor cells, ex- press high levels of the verapamil-sensitive multidrug-resistance membrane efflux pump (P-glycoprotein), which confers resistance to multiple chemotherapeutic agents. This pump also excludes cer- tain fluorescent dyes, such as rhodamine 123 or Hoechst 33342. By using these dyes in combination with flow cytometry, it has been possible to identify a population of haematopoietic cells with low dye retention, so-called side population (SP) cells. Although this population is markedly enriched for HSCs, SP cells still represent a heterogeneous mix and are not equivalent to pure HSCs. While the SP phenotype has been useful in characterizing murine HSCs, this characteristic has not translated as easily into the human system. Though HSPC populations as currently defined are still impure these methods have allowed isolation of cell populations of defined functionality and are useful to identify genes and signalling pathways that mediate human HSPC differentiation. Isolation of distinct HSPC populations is also beginning to permit careful dissection of the hier- archical relationships between different blood cell populations: an essential step in describing the cellular basis of normal haematopoi- esis. In turn, this is critical when trying to understand (1) the normal cellular compartments where genetic and epigenetic changes initially occur in haematological diseases (i.e. the disease initiating cell popu- lations); (2) the cell compartments where subsequent mutations/ epigenetic change is acquired during disease evolution and how this changes the haemopoietic hierarchy; and (3) the cell populations that propagate haemopoietic disease. Advances in cell sorting, gen- etic analysis, and single-cell biology are making analysis of HSPCs increasingly precise. This progress will certainly provide additional insight into HSC biology and heterogeneity in the near future. In routine clinical transplantation practice, isolation of HSCs to high purity is not necessary. Safe transplantation is routinely possible with both unfractionated mononuclear cells and CD34+ purified grafts (usually by magnetic beads that enrich CD34+ cells to c.60– 85% purity). Indeed, transplantation of highly purified HSCs may delay engraftment, as initial engraftment in patients is most likely from progenitors rather than HSCs. Nevertheless, there may be merit in transplanting purified HSPCs in some clinical situations—for ex- ample, to reduce transplantation of contaminating tumour cells in autologous transplantation. In this regard, there are encouraging clin- ical studies where transplantation of CD34+Thy1(CD90)+ cells has been employed. However, this requires a flow cytometry-based iso-

lation procedure that is difficult to implement on a widespread basis. Pluripotent stem cells and haematopoiesis Mouse embryonic stem cells (mESCs) were first isolated in 1981. mESCs have proven invaluable for studies of basic mammalian developmental biology, including haematopoietic development. Unlike adult stem cells (such as HSCs), ESCs are able to undergo self-renewal indefinitely in culture, yet maintain the ability to form all somatic cell lineages (including haematopoietic cells). Studies with mESCs have been crucial to identifying genes that regulate haematopoietic development, through gene deletion and/or ma- nipulation. Notably, attempts to derive HSCs capable of long-term multilineage engraftment have largely only been successful when mESCs have been genetically manipulated by overexpression of spe- cific transcription factors, for example, HoxB4. The first description of human ESCs was in 1998. Like mESCs, hESCs can be maintained indefinitely as a self-renewing popu- lation in culture, yet maintain the ability to form all somatic cell populations. hESCs have also been used to investigate human haematopoiesis and have generated considerable interest because of their potential to produce large numbers of human cells and tissues suitable for studying disease mechanisms, transplantation, or transfusion medicine. For example, there has been consider- able interest in using hESCs to produce red blood cells or platelets as an adjunct to the supply from blood donation. Additionally, the potential to produce HSCs from hESCs is of great interest. To date, however, although most mature blood cell populations have been produced from hESCs, it has not been possible to iso- late HSCs to any reasonable extent. Even genetic manipulation and overexpression of transcription factors, such as HoxB4, has not been similarly effective in the human system. Considerable ef- forts to identify strategies to improve development of HSCs from hESCs are ongoing. Another important cell type is the induced pluripotent stem cell. iPSCs can be derived from various somatic cell populations, typic- ally by expression of a limited number of ‘reprogramming genes’ that are able to convert the somatic cell population into cells that function like embryonic stem cells. These studies were first per- formed in mouse cells in 2006 and then from human cells in 2007. Like their ESC counterparts, iPSCs have been used to derive diverse haematopoietic cell lineages. Again, to date, transplantable HSCs have not been derived from iPSCs. This field will continue to mature, and there is considerable interest in deriving iPSCs from individuals with genetic deficiencies to model genetic disease. Using iPSCs, gene correction strategies or other means to overcome the genetic defect can be analysed. This may lead to effective therapies based on using iPSCs as a screening resource and would not require direct trans- plantation of iPSC derived cells. Additionally, future developments may allow for derivation of iPSCs from individuals with haemato- logical or other diseases and use of these cells to produce essentially autologous replacement cell populations. Haematopoietic niche In the adult, haematopoietic cell differentiation from HSPCs is regu- lated by signals provided by the BM microenvironment. The spe- cific cellular constituents of the microenvironment that influence blood cell development are still being elucidated. They include

SECTION 22 Haematological disorders 5178 mesenchymal cells, endothelial and neural cells, haematopoietic cells, and extracellular matrix. The heterogeneous mesenchymal stromal cell (MSC) population plays a significant role in the haem- atopoietic niche. Some MSCs are part of the continuum of cells that produce bone and some are perivascular without a clear role in skel- etal biology. Both of these cell types influence haematopoiesis. For example, mature osteoblasts are important in stem cell mobiliza- tion, nestin-positive mesenchymal stem cells are important for HSC persistence, and adipocytes have been implicated as negative regu- lators of HSC number. In human studies, cord blood co-cultured with MSCs undergo a median 30-fold expansion of CD34+ cells resulting in significantly improved engraftment. In mouse, leptin receptor-expressing (LepR+)

cells represent the major proportion of MSCs. LepR+ cells appear to be the main source of new osteo- blasts and adipocytes in adult bone marrow and form bony ossicles supportive of haematopoiesis in vivo. LepR+ MSCs are the major source of the cytokines stem cell factor (SCF) and chemokine (CXC motif) ligand 12 (CXCL12) in mouse bone marrow. Conditional deletion of the stem cell factor gene (Scf) in LepR+ cells leads to depletion of quiescent HSCs and conditional deletion of the gene encoding CXCL12 (Cxcl12 also called Sdf1) in LepR+ cells leads to HSC mobilization. Other cell types, such as neural cells of the sympathetic nervous system and nonmyelinated Schwann cells, also support HSCs. The sympathetic nervous system mediates circadian modulation in the number of HSCs moving from BM to bloodstream on a daily basis. Mature haematopoietic cells are also thought to influence HSC function in the BM. Specifically, macrophages help regulate HSC mobilization into blood and T cells are thought to influence HSC engraftment and provide relative protection from immune attack. Megakaryocytes have been shown to be important for maintaining HSC quiescence, with various megakaryocyte-secreted factors, including CXCL4, transforming growth factor-β1 (TGFβ1), and thrombopoietin, implicated in this role. Therefore, a complex admixture of cells participates in what is designated as the stem cell niche (Fig. 22.2.1.6). sinusoidal endothelial stem cell niche HSC HSC self- renewal stem cell endothelial niche — spindle-shaped osteoblast cells non-stem cell niche environmental asymmetry MPP differentiation divisional asymmetry – Myh CXCL4 – osteopontin niche players N-cadherin HSC SNO cells TIE2 ANG-1 CXCL12 CXCR4 2 Ca sensory receptor 3 SDF1 CXCL 12 receptor CXCR4 bone 4 Notch/Jagged 5 BMP 6 ICAM-1 7 N-cadherin 1 Osteopontin (a) (b) Fig. 22.2.1.6 (a) The bone marrow niche, which, in part, consists of sinusoidal endothelial cells, helps control haematopoietic stem cell (HSC) fate. HSCs can be quiescent (G0 of cell cycle) or can enter the cycle to divide symmetrically or asymmetrically (divisional asymmetry) to self-renew and/or to produce more differentiated cells such as multipotential progenitors (MPPs). HSCs can also migrate into and out of the niche (environmental asymmetry). The components of the niche are shown below. (b) This shows an HSC anchored into the niche via binding of: (i) cell surface receptor TIE-2/TEK binding to its ligand angiopoietin-1 (ANG-1) on sinusoidal endothelial cells (SNO cells) and (ii) the CXC-chemokine ligand 12 on SNO cells binding to its receptor CXCR4.

22.2.1 Cellular and molecular basis of haematopoiesis 5179 The niche serves several functions important for haematopoi- esis. The first is the regulation of stem cell self-renewal, a process that requires expression of molecules such as SCF and members of the WNT family. The second is control of stem cell number. The third is the coordinated regulation of proliferation and differenti- ation of HSCs. When the niche is perturbed in mice, it can lead to myeloproliferative or myelodysplastic phenotypes. The fourth is cell localization, a process that is important in the context of either harvesting stem cells by mobilization into the blood or delivery of transplanted HSCs to enable engraftment. Thus, the HSC niche is a critical aspect of the regulated pro- duction of blood cells throughout life. Unravelling how stem cells enter and leave the niche will lead to improved methods to mobilize stem cells for clinical harvest (see ‘HSPC circulation, homing, and mobilization’). Ongoing efforts to improve stem cell engraftment into the niche and to discern how the niche contrib- utes to disease may contribute to future manipulation of the niche for clinical benefit. Regulation of haematopoietic differentiation A complex network of transcription factor and growth factor signalling pathways regulates HSPC self-renewal, lineage com- mitment, and differentiation. Transcription factors (TFs) that are expressed either exclusively in blood cells, or have restricted tissue- specific patterns of expression, play important roles in regulating blood

production. Furthermore, acquired driver mutations of these TFs are pathogenic in haematological diseases such as lymphoma and leukaemia. The importance of these TFs is also underscored by the conserved role they play in haematopoiesis through evolution. Over the last two decades, this attribute has allowed the function of these TFs to be extensively investigated in animal models. In these models, genes encoding critical TFs have been deleted, modified, overexpressed, and misexpressed. A summary of the site of action of some of these TFs is shown in Fig. 22.2.1.7. A thorough description of the function of these proteins is not possible here. Key points that arise from these studies are as follows: RUNX1 SCL LMO2 ETV6 GATA2 GATA2 GATA2 GATA1 ZFPM1 KLF1 SCL MYB BCL11A STAT5 GATA2 GATA1 ZFPM1 ETS Factors RUNX1 SCL MYB GATA2 GATA1 STAT5 GATA 1 CEBPE STAT3 GFI1 PU.1 CEPBA CEBPE TCF3 IKAROS E2A PU.1 EBF PAX5 BLIMP1 PU.1 CEPBA CEBPE IRF 8 EGR1/2 E2A IZKF NOTCH GATA3 RUNX1 TCF-1 BCL11B HEB MYB B-cells Monocytes Neutrophils Eosinophils Mast cells Megakaryocytes Red cells HSC in development HSC in adult life T-cells Fig. 22.2.1.7 A schematic representation of the key haematopoietic-specific transcription factors (in boxes) required for specification and/or maturation of different haemopoietic lineages. Thus, for example, the transcription factors GATA2, RUNX1, SCL, LMO2, and ETV6 are all required for specification of HSCs in development. GATA2 is required for maintenance of HSCs in adult life. Transcription factors required for each of eight mature blood lineages are shown.

SECTION 22 Haematological disorders 5180 • TFs are divided into families that have similar proteins domains. • They often bind DNA and interact with other proteins (other TFs and proteins that control transcription) via specific domains. • TFs work in combinations to both activate and

repress the expres- sion of a large number of genes. • TFs are required at discrete stages of haematopoiesis and any one TF often functions at multiple stages within one lineage and can function in more than one lineage. • Ultimately, TFs work in complicated networks that can be mod- elled much like semiconductor/computing networks. TFs work in negative feedback loops, feed-forward loops, and cross- antagonistic loops to mention just three such types of interaction. • The function

of TFs helps that regulate the cell’s potential to make blood cells of different lineages, proliferate, undergo apoptosis and self-renew. More specifically, the TFs SCL/TAL1 and LMO2 are required to specify HSC from mesoderm. The TFs RUNX1 (AML1), TEL1, MLL, and GATA2 are required to maintain stem cells once they have been specified. In myelopoiesis, the TFs PU.1, the C/EBP family (C/EBPα and C/EBPε), GFI-1, EGR-1, and NAB2 all promote the granulocyte-macrophage lineage programmes. GATA2 is required in stem/early

progenitor cells but is also required for mast cell dif- ferentiation and in the early phases of megakaryocyte–erythroid lineage maturation. Working with GATA2 to promote erythropoi- esis and megakaryopoiesis are GATA1, FOG1, SCL, EKLF, p45NF- E2, and FlI-1. In early lymphopoiesis, the TF Ikaros is required. In B-lymphopoiesis, the TFs E2A (and its family members), EBF, and PAX5 are required and finally the TF BLIMP1 is necessary for plasma cell formation. In T-cell maturation,

notch signalling acti- vates the TF CSL, which works with the TFs GATA3, T-BET, NFATc, and FOXP3. Of note, the TFs SCL/TAL1, MLL, RUNX1, LMO2, PU.1, C/EBPa, PAX5, E2A, and GATA1 are all implicated in the pathogenesis of human leukaemia. In addition to TFs, transcription is also controlled at the level of accessibility of DNA that is packaged in chromatin in the cell nu- cleus. A large number of proteins regulate accessibility by reversibly altering DNA methylation and modifications of histone that package DNA. These

alterations in packaging alter gene expression without changing the DNA sequence and are known as epigenetic changes. Protein expression is also regulated by alternative RNA splicing that alters the structure of the translated protein. Genes encoding epi-genetic regulators and RNA splicing factors are recurrently mutated in blood cancers, underscoring their importance in haematopoietic differentiation. Some of the epigenetic regulators mutated in blood cancers are shown in Fig. 22.2.1.8. HSPC

circulation, homing,
and mobilization HSPCs migrate from one site of blood cell production, enter the circu- lation, home, and enter other supportive sites. This certainly is the case in development before the existence of bones in the fetus. HSPCs even- tually transit from fetal liver to nascent BM to establish haematopoi- esis via the bloodstream. Even once haematopoiesis becomes restricted to the bone marrow, HSPCs traffic into and out of the BM regularly. Experiments using

parabiotic mice, in which the circulations of two separate mice are joined surgically, have indicated that murine HSCs exit the BM and transit through the peripheral blood system at surprisingly high flux rates (estimated to be c.104–105 long-term repopulating HSCs per day in a mouse). Other mouse studies have shown that macrophages and osteoblasts are critical for granulocyte colony-stimulating factor (G-CSF)-mediated effects on HSPC trafficking through regulation of the SDF-1–CXCR4 axis (see later in

this section). This ability of HSPCs to move from the BM to the peripheral bloodstream is exploited for collection of stem cells for clinical haematopoietic cell transplantation. The rate, timing, and destin- ation of the HSPCs that circulate from the BM to the periphery involve chemokines and their receptors, especially CXCL12 (or SDF-1) and its receptor CXCR4; integrins, particularly very late antigen-4 (VLA-4, also termed integrin α4β1); selectins, such as P- selectin glycoprotein ligand-1 (PSGL1, also

termed CD162); and the calcium-sensing receptor and the intracellular-signalling molecules, Gαs and Rac1/Rac2. CXCL12/SDF-1 is produced by several marrow stromal cell types. These include immature osteoblasts located in the endosteal region adjacent to stem cell niches and the endothelium. The SDF-1 receptor, CXCR4, is expressed on a wide variety of haematopoietic cells, and is important in stem cell self-renewal IDH1/2 TET2 DNMT3A EZH2 Trithorax proteins MLL HATs HDACs ASXL1 Polycomb repressive

complex 2 (PRC2) hydroxymethylcytosine methylcytosine H3K27me3 H3K4me3 H3/H4 Ac PRMT5 H2A/H4me + +

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Fig. 22.2.1.8 Schematic representation of DNA (orange) wrapped around histone octamers (blue discs). DNA can be reversibly methylated on cytosine (green hexagon) by the DNA methyltransferases (DNMTs). Demethylation of DNA occurs via intermediates that include hydroxymethylcytosine (blue octagon). Demethylation requires the TET2, IDH1, and IDH2 proteins. Histones have peptide tails that protrude away from the histone octamer (shown as brown lines). Amino acids in these tails can be post-translationally modified. Examples of these modifications include (1) acetylation (Ac) at histones H3 and H4 (shown by orange ball), by histone acetyltransferases (HATs). Acetyl marks can be removed by histone deacetylases (HDACs). (2) Methylation on lysine (H3K27—purple ball and H3K4—blue ball) or arginine (H2A and H4 purple ball). The H3K27 methylation is mediated, in part, by the polycomb repressive complex 2 (PRC 2). H3K4 methylation is mediated by the trithorax protein family, which includes MLL as a member. One of the arginine methyltransferases is PRMT5. Epigenetic changes associated with gene activation include histone acetylation, H3K4 trimethylation, and DNA demethylation. Proteins mediating these changes are shown in a blue font. Conversely, epigenetic changes associated with gene repression include H3K27 trimethylation, arginine methylation, and DNA methylation. Proteins mediating these changes are shown in a red font.

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