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Bone Marrow Failure

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Preface

For many years, bone marrow failure was a pathological entity, all thrown into the same bin without thought to pathogenesis, diagnosis, prognosis, and, certainly, treatment. Forward to the age of blood banking, advanced pharmacology, imaging, and other trappings of modern medicine, the full flower of the diversity of these diseases became apparent. Now, in 2018, with DNA sequencing occurring at the highest technical level, cheapest price, and overwhelming volume, a blizzard of genes that account for these varying syndromes has come into focus, with the science of investigating the function of these genes following close on the heels of this information.

In this volume we lay out a compendium of chapters outlining the bone marrow failure syndromes. Rather than proceeding in an encyclopedic way, importantly we have elected to follow the science. In particular, we start with a discussion of basic molecular hematopoiesis to obtain an understanding of the normal processes that go awry during development. In particular are genes that not surprisingly are mutated in some of the diseases are explored in these pages. Further we find threads of connection among seemingly disparate syndromes Diamond-Blackfan anemia, dyskeratosis congenita, and Shwachman-Diamond, all linked by the understanding of ribosome biology. While bone marrow failure classically invokes all three cell lines, the effects of genetic mutation in individual cell lines are apparent, as in the neutropenias and thrombocytopenias. The culmination of bone marrow failure is symbolized in the continuous shrinkage of the "idiopathic" aplastic anemias, as new genes are identified in the pathogenesis of bone marrow failure, and the joining of the circle with myelodysplasia, as inevitably entering into a Venn diagram together. Most compellingly is the emergence of GATA2 as the gene responsible for MDS. This is a reminder that, while our mission is certainly paramount to those patients afflicted with these rare diseases, these diseases are important and profound genetic models of normal biology. Perhaps no better example of this exists than Fanconi anemia, which not only lends insight into DNA repair but also into the genetics of common cancers, as 15–20% of all cancers in the cancer genome database contains mutations in FA genes or in related genes. Thus, rare has become mainstream.

Finally, as is recognized by many of us who care for these patients, the current state of the art of therapy remains hematopoietic stem cell transplant, with much research devoted to the safe and effective provision of such ther-

apy. Current limitations and the promises of the future portend going further and suggest emerging therapies that will capitalize on new genomic technology.

May the efforts of all invested in scientific efforts to understand these diseases and those who care for the patients so afflicted, clinicians and families, be rewarded swiftly for their labor, perseverance, and devotion to good works.

New Haven, CT — Gary M. Kupfer
Washington, DC — Gregory H. Reaman
Cincinnati, OH — Franklin O. Smith

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To the authors, scientists, clinicians, families, and our patients, our appreciation and admiration for your sense of duty in your life's work.

Our love and appreciation to our spouses, Michele, Phyllis and Susan, and our children, Noa, Zev and Meital, Barrett and Ian, and Emily and Sarah.

Contents

The Cellular and Molecular Mechanisms of Hematopoiesis

1

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1.1 Hematopoiesis

Hematopoiesis is a complex and dynamic process where mature blood cells of the myeloid and lymphoid lineages are produced from a common hematopoietic progenitor cell. In adult mammals, this process occurs almost exclusively in the bone marrow under homeostatic conditions. This hierarchal process begins with a common hematopoietic stem cell (HSC) that is multipotential and capable of forming all blood lineages while maintaining their self-renewal capacity. In the classical model of hematopoiesis, it is thought that HSCs differentiate into a series of progenitor cell intermediates that undergo gradual fate restriction and commitment into mature blood lineages (Figs. 1.1 and 1.2, (Doulatov et al. 2012)). Recent advances in single-cell tracing, RNA sequencing, and transplantation have suggested an alternative model where lineage fate may be determined at an earlier stage than previously suggested. Single-cell RNA sequencing on myeloid progenitor cells revealed that very few

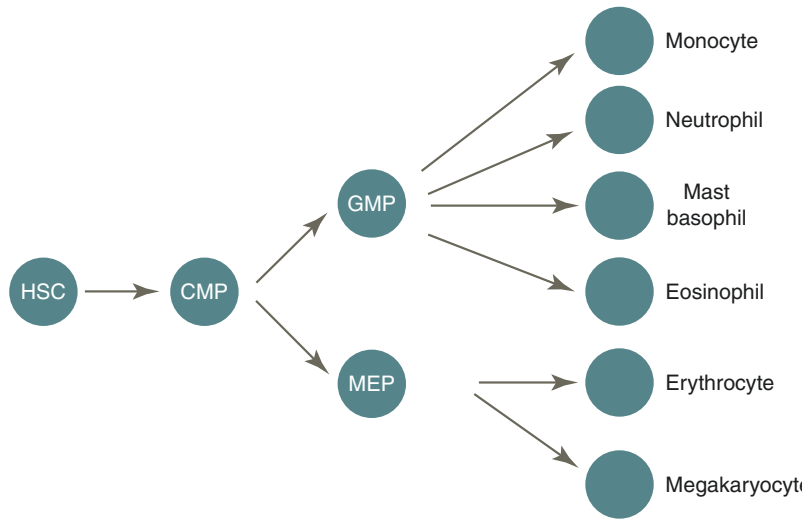

Fig. 1.1 Classical model of myeloid lineage commitment in the bone marrow. In the classical model of hematopoiesis, mature myeloid cells are generated through a series of myeloid progenitors including a common myeloid progenitor (CMP), granulocyte/macrophage precursor (GMP), and a myeloid/erythroid precursor. Recent studies indicate that myeloid specification may occur at an earlier stage where myeloid progenitors are a mix of committed progenitors rather than a homogenous population of cells with multilineage potential

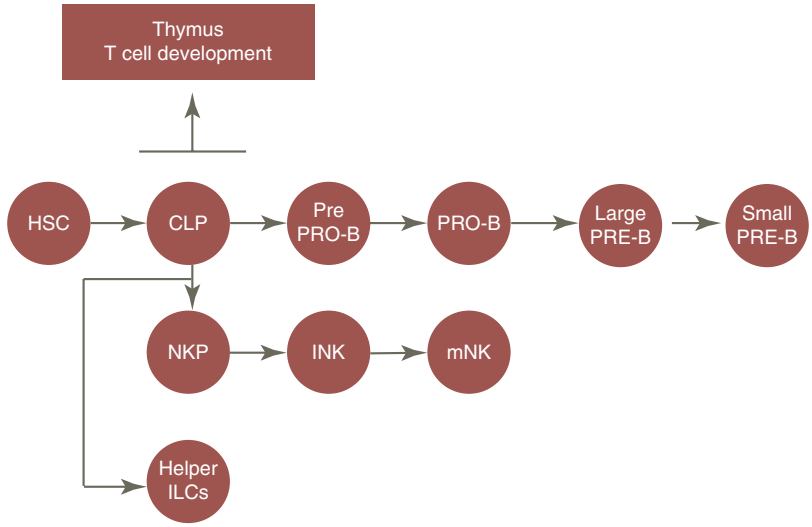

Fig. 1.2 Lymphoid lineage commitment in the bone marrow. During lymphoid develop, the common lymphoid progenitor is generated from hematopoietic progenitors that give rise to natural killer cell, B cell, and helper innate lymphocyte cell progenitors within the bone marrow. B cell and NK cell progenitors go through stages of maturation before leaving the bone marrow, whereas T cell progenitors mature within the thymus

progenitors expressed transcription factors that regulate multiple cell fates. Instead, individual cells clustered into lineage-specific differentiation programs (Paul et al. 2015). Additionally, single-cell lineage tracing through the transplantation of barcoded progenitor cells into mice suggested that very few myeloid progenitor cells have the ability to form erythroid and myeloid lineages suggesting that myeloid progenitors are a mixture of committed progenitors rather than a homogenous population of cells with multilineage potential (Perie et al. 2015). The regulation of HSC production, survival, self-renewal, and differentiation is a carefully orchestrated process that is dependent upon both systemic and local cues to produce approximately one trillion (10^{12}) cells daily. In this chapter, we will review the cellular intrinsic and extrinsic factors that control adult hematopoiesis in the bone marrow microenvironment.

1.2 HSCs

Hematopoietic stem cells (HSCs) are multipotent progenitors capable of forming all blood lineages while maintaining their self-renewal capacity. HSCs are extremely rare as only 1 in a million cells within human bone marrow is a transplantable stem cell (Doulatov et al. 2012). The isolation and purification of murine HSCs in combination with genetic approaches have been informative in revealing the molecular and biochemical pathways that control HSC function and differentiation.

1.2.1 Transcription Factors

There are a variety of transcriptional networks that control HSC production, survival, and self-renewal. Genetic inactivation of a number of these factors has identified important roles for Gata-2, Notch1, Erg, N-Myc and c-Myc, and Gfi-1 in the regulation of hematopoiesis in the bone marrow.

The zinc finger transcription factor GATA-2 is required for both primitive and definitive hematopoiesis. Germline inactivation of GATA-2 leads to embryonic lethality at E10.5 due to severe anemia (Tsai et al. 1994). The injection of GATA-2-deficient/wild-type ES cell clones into blastocysts revealed that GATA-2 is required for fetal liver and adult bone mar-

row hematopoiesis (Tsai et al. 1994). GATA-2 expression is decreased in CD34+ bone marrow cells in patients with aplastic anemia underscoring the importance of this transcription factor in the regulation of hematopoiesis (Fujimaki et al. 2001).

Notch1 is a membrane-bound receptor and transcription factor whose activity is regulated by interaction with its ligand Jagged. Upon ligand interaction, Notch1 is proteolytically cleaved to release the Notch-IC domain that translocates into the nucleus to regulate transcription. Notch1 is required for definitive hematopoiesis as Notch1-deficient ES cells fail to contribute to long-term definitive hematopoiesis (Hadland et al. 2004). A key mechanism by which Notch1 regulates hematopoiesis is through the regulation of Runx1 (Wilkinson and Gottgens 2013).

The E-twenty-six (ETS) transcription factor-related gene (ERG) plays an important role in the expansion and maintenance of HSCs during the early phases of definitive hematopoiesis. Conditional deletion of ERG in hematopoietic cells resulted in loss of myeloid cells associated with a defect in HSC numbers and function in transplantation assays (Knudsen et al. 2015). While HSC migration, homing, and adhesion are not dependent on ERG activity, ERG is required to maintain and prevent the differentiation of HSCs in the bone marrow. ERG-dependent regulation of HSCs is associated with the activation of HSC self-renewal genes and the repression of MYC target genes (Knudsen et al. 2015). The DNA-binding activity of ERG may be particularly important in the regulation of definitive hematopoiesis as $ERG^{Mld2/Mld2}$ DNA-binding mutant mice also fail to maintain HSCs in the bone marrow of adult mice (Taoudi et al. 2011).

The basic helix-loop-helix leucine zipper transcription factors N-Myc and c-Myc play an important role in controlling HSC proliferation, differentiation, and survival. Immature HSCs express both N-Myc and c-Myc. Conditional deletion of c-Myc in adult bone marrow cells results in the accumulation of HSCs that are impaired in differentiation (Wilson et al. 2004). While conditional inactivation of N-Myc did not affect steady-state hematopoiesis, the combined deletion of N-Myc and c-Myc resulted in severe pancytopenia in mice associated with a preferential loss of proliferating, but not quiescent HSCs (Laurenti et al. 2008).

The zinc finger repressor growth factor independent-1 (Gfi-1) restricts the proliferation and preserves the functional integrity of HSCs. Gfi-1-deficient HSCs display elevated proliferation rates but are functionally compromised in competitive repopulation and serial transplantation assays (Hock et al. 2004; Zeng et al. 2004).

1.2.2 Epigenetics

Epigenetic modification of chromatin and DNA plays an important role in the regulation of HSC function. A number of factors involved in DNA methylation, histone modification, and chromatin remodeling have been identified as key regulators of HSC development, self-renewal, and differentiation (for a recent review, see (Cullen et al. 2014)). Large-scale genetic screens in zebrafish have been utilized to identify multiple chromatin-modifying complexes important in hematopoietic development (Huang et al. 2013). Among these factors, the polycomb-group proteins (PRC1 and PRC2) mediate gene silencing through the methylation of histones. BMI1, a component of PRC1, maintains the self-renewal of adult stem cells through the repression of the Ink4a/Arf locus (Oguro et al. 2006). Moreover, BMI1 prevents premature lineage specification of HSCs by repression of developmental regulator genes Ebf1 and Pax5 (Oguro et al. 2010). Recent studies have identified a role for the histone deacetylase SIRT6 in the regulation of HSC homeostasis through the transcriptional repression of Wnt target genes (Wang et al. 2016). Sirt6 deletion in HSCs promoted proliferation and impaired self-renewal ability through the aberrant activation of Wnt signaling (Wang et al. 2016). Additionally, DNA methylation mediated by DNMT3A and TET2 plays an important role in repressing lineage-specific factors such as Klf1 in HSCs. Inactivation of DNMT3A and TET2 commonly occurs in hematological malignancies. In murine models, Tet2 and Dnmt3a inactivation leads to

myeloid and lymphoid disease that is associated with Klf1- and Epor-enhanced HSC self-renewal (Zhang et al. 2016).

1.2.3 Microenvironment

The bone marrow is a complex and dynamic environment with multiple "niches" that control HSC quiescence, self-renewal, and proliferation. The niche provides a variety of signals including growth factors, cytokines, oxygen gradients, cell adhesion molecules, matrix proteins, and nutrients that control HSC function (Fig. 1.3). In particular, CXCL12 plays an important role in HSC retention and quiescence. Global deletion of CXCL12 or its receptor, CXCR4, results in HSC loss in the bone marrow. In addition to CXCL12, the Notch, Wnt/B-catenin, SCF/c-Kit, TPO/c-Mpl, TGF-B/Smad, and Ang-1/Tie2 signaling pathways have been implicated in the regulation of HSC function (for a recent review, see (Kosan and Godmann 2016; Seita and Weissman 2010)).

Research over the past decade has identified a number of cell types within the bone marrow microenvironment that support HSC function. Osteoblasts were the first cell population shown to support HSC frequency in the bone marrow (Calvi et al. 2003). Conditional expansion and deletion of osteoblasts were sufficient to modulate HSC numbers in the bone marrow (Calvi et al. 2003; Zhang et al. 2003). In addition to osteoblasts, endothelial cells, mesenchymal stromal cells, macrophages, osteoclasts, and non-myelinating Schwann cells have been shown to promote HSC maintenance in the bone marrow. The cells within the niche produce a variety of factors that regulate HSC function including SCF, CXCL12, pleiotrophin, Slit2, tenascin-C, osteopontin, and noncanonical Wnts (for a recent review, see (Morrison and Scadden 2014)). Recent technological advances in single-cell RNA sequencing are being employed to identify novel niche factors that contribute to HSC function in the bone marrow. Using these approaches, Silberstein et al. identified the secreted RNase angiogenin, the cytokine IL-18, and the adhesion molecule Embigin as novel factors produced by mesenchymal cells that regulate HSC quiescence (Silberstein et al. 2016). In summary, the bone marrow microenvironment contains many cellular and molecule factors that control HSC function (Fig. 1.3).

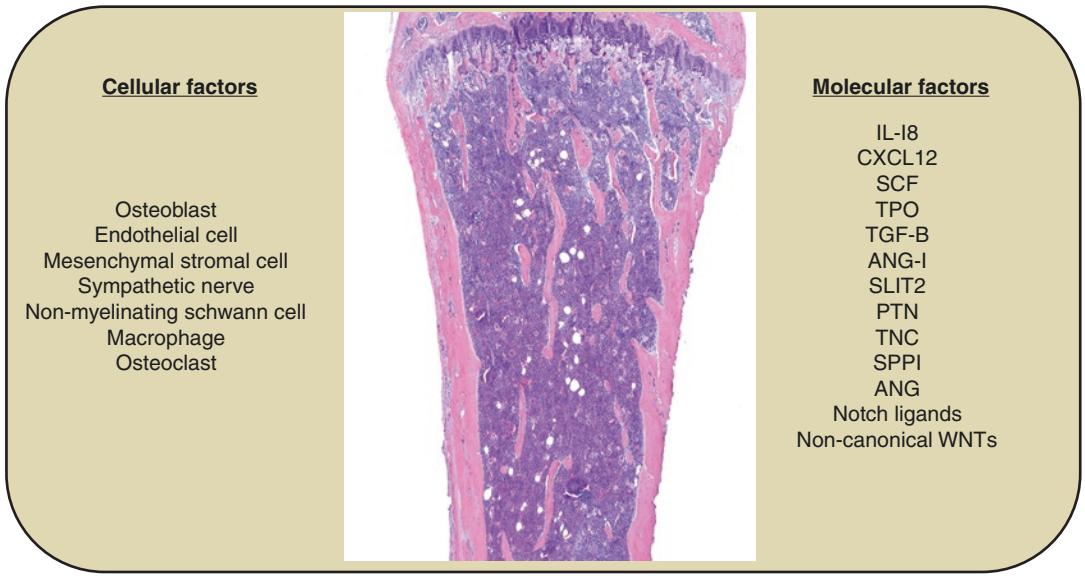

Fig. 1.3 Cellular and molecular factors within the HSC niche that control HSC function. Recent studies have demonstrated that the bone marrow microenvironment is highly complex and dynamic with multiple cellular and molecular factors contributing to the regulation of hematopoiesis

1.3 Erythrocyte

Erythropoiesis is the process by which mature red blood cells are produced from hematopoietic stem and progenitor cells in the bone marrow. Red blood cells are anucleate cells that play a critical role in carrying oxygen to tissues. Red blood cells are the most abundant cells in the blood (5 million/μL). In order to maintain RBC numbers in the blood, the bone marrow produces two million erythrocytes per second. At this level of production, intrinsic and extrinsic factors controlling erythropoiesis must be carefully coordinated (Palis 2014). In the bone marrow, erythroid precursors differentiate through a series of stages (proerythroblasts, basophilic and polychromatophilic erythroblasts, orthochromatophilic erythroblasts, and finally reticulocytes by enucleation) that have been defined morphologically, based on a gradual decrease in cell volume, increasing chromatin condensation and increasing hemoglobinization as well as by the expression of the cell surface molecules CD71 and Ter119 (Socolovsky et al. 2001).

1.3.1 Transcription Factors

The complete mechanisms that control erythropoiesis remain to be elucidated. However, there are a number of transcription factor complexes that have been shown to control erythroid gene expression programs in adult hematopoiesis.

The zinc finger transcription factor, GATA-1, plays an important role in erythroid development and maturation (Pevny et al. 1991). GATA-1 is expressed early in erythropoiesis and is required for the maturation of proerythroblasts (Pevny et al. 1995). GATA-1-deficient embryonic stem cells fail to give rise to mature red blood cells (Pevny et al. 1991). Together with additional transcriptional regulators including friend of GATA-1 (FOG-1), TAL1, IRF2, IRF8, LMO2, and LDB1, GATA-1 represses and activates target genes important for erythropoiesis.

In addition to GATA-1, the erythroid-specific Kruppel-like factor KLF1 is essential for erythropoiesis. KLF1 expression is restricted to erythroid cells where it plays an essential role in regulating erythroid-specific gene expression. Genetic inactivation of KLF1 in mice results in embryonic lethality associated with anemia and molecular features of B-globin deficiency (Perkins et al. 1995). In addition to regulating B-globin expression, KLF1 activates the expression of genes that control heme synthesis, globin chaperones, structural membrane and cytoskeletal proteins, ion and water channels, metabolic and antioxidant enzymes, and cell cycle regulators (Perkins et al. 2016). The importance of KLF1 in the regulation of erythropoiesis is underscored by recent identification of KLF1 variants in patients with red blood cell disorders (Perkins et al. 2016).

1.3.2 Growth Factor Signaling

The glycoprotein erythropoietin (EPO) is essential for the regulation of red blood cell mass in response to changes in tissue oxygenation. EPO induces erythropoiesis through the stimulation of the EPO receptor on erythroid precursor cells to stimulate survival, proliferation, and differentiation, thus enhancing the oxygen-carrying capacity of blood (review in ref. (Ebert and Bunn 1999)). Lack of Epo during murine development results in embryonic lethality at E13.5 as a result of cardiac failure and anemia (Wu et al. 1999; Wu et al. 1995). Clinically, dysregulated *EPO* expression results in the development of anemia when serum EPO levels are inadequately low or polycythemia as a result of EPO overproduction. *EPO* expression is tightly regulated by developmental, tissue-specific, and physiological cues (Ebert and Bunn 1999). In adult mammals, the kidney is the primary physiologic source of EPO (Zanjani et al. 1981). The primary physiological stimulus of increased *EPO* gene transcription is tissue hypoxia, which can induce up to a 1000-fold increase in circulating serum EPO levels (Ebert and Bunn 1999). EPO/EPOR signaling promotes erythroid development in large part through the activation JAK2 signaling. Inactivation of JAK2 results in the development of anemia, whereas constitutive JAK2 mutations lead to increased

red blood cell mass and polycythemia (Neubauer et al. 1998; Levine et al. 2005).

1.3.3 Microenvironment

Within the bone marrow microenvironment, erythroblasts mature within erythroblastic islands where erythroblasts physically attach to a central macrophage. Macrophages are thought to promote erythroblast proliferation, supply iron for hemoglobin, promote enucleation, and clear nuclear debris (for a recent review, see (Giger and Kalfa 2015)). An important functional role for bone marrow macrophages in mediating erythroblast survival under stress has been described (Korolnek and Hamza 2015; Chow et al. 2013). Future studies are needed to determine the molecular mechanisms by which central macrophages support erythropoiesis.

1.4 Megakaryocyte

Platelets are the second most abundant cell type in the blood and play an important role in hemostasis, thrombosis, inflammation, and vascular biology. Platelets are anucleate cells that are derived from mature megakaryocytes in the bone marrow (Kaushansky 2008). A single megakaryocyte can give rise to 1000–3000 platelets (Kaushansky 2008). The adult human produces 10^{11} platelets daily at steady state (Kaushansky 2008). Platelets have an average lifespan of 9 days once they enter the circulation. Both cellular intrinsic and extrinsic factors control thrombopoiesis.

1.4.1 Transcription Factors

Platelets are generated in the bone marrow through a process of megakaryocyte differentiation and the fragmentation of the megakaryocyte cytoplasm. Megakaryocytes are derived from pluripotent HSCs that undergo differentiation to a common myeloid progenitor (CMP) and a bipotent megakaryocyte/erythroid progenitor (MEP) cell. There are a number of transcription factors that orchestrate megakaryocyte differentiation and platelet production within the bone marrow including GATA-1, friend of GATA-1 (FOG), NF-E2, Fli-1, PU.1, and RUNX1.

The zinc finger transcription factor GATA-1 is a key regulator of erythroid and megakaryocyte differentiation and maturation. In megakaryocyte production, overexpression of GATA-1 in an early mouse myeloid cell line induces megakaryocyte differentiation (Visvader et al. 1992). Moreover, genes that are expressed within the megakaryocyte lineage depend on GATA consensus sites for full transcriptional activity (Shivdasani et al. 1997). In mice, conditional inactivation of GATA-1 in megakaryocytes significantly reduces megakaryocyte proliferation and cytoplasmic maturation (Shivdasani et al. 1997). Moreover, patients with inherited mutations within *GATA-1* present with platelet dysfunction and thrombocytopenia (Daly 2017). The functional role of GATA-1 in megakaryocytes is to recruit transcriptional cofactors, such as friend of GATA-1 (FOG) to megakaryocyte-expressed genes. Genetic studies have demonstrated that the interaction between GATA-1 and FOG1 is essential of megakaryocyte differentiation (Wang et al. 2002; Tsang et al. 1997).

Runt-related transcription factor (RUNX1 or AML1) is essential for definitive hematopoiesis and plays an important role in megakaryocyte differentiation. Germline inactivation of *Runx1* in mice is embryonically lethal due to severe hematopoietic defects. Moreover, hematopoietic-specific inactivation of RUNX1 in adult mice results in abnormal megakaryopoiesis and thrombocytopenia (Ichikawa et al. 2004). Clinically, mutations or inactivation of RUNX1 observed in patients is associated with leukemia, myelodysplastic syndromes, and a familial platelet disorder with a predisposition to acute myeloid leukemia (Daly 2017). An important mechanism by which RUNX1 controls megakaryocyte differentiation is through the downregulation of non-muscle myosin IIB heavy chain (MYH10) that contributes to megakaryocyte polyploidization (Lordier et al. 2012). MYH10 expression in platelets is a biomarker for RUNX1 and FLI1 alterations in patients with platelet disorders and myeloid neoplasms (Antony-Debre et al. 2012).

The basic region leucine zipper transcription factor NF-E2 plays a critical role in platelet formation. NF-E2 is a heterodimer that is comprised of a hematopoietic restricted 45 kDa (p45) subunit and a widely expressed 18 kDa (p18) subunit. Deletion of the p45 subunit in mice leads to absolute thrombocytopenia and mortality from hemorrhage. While NF-E2 inactivation does not affect megakaryocyte proliferation in response to TPO, NF-E2-deficient megakaryocytes fail to form platelets in their cytoplasm (Shivdasani et al. 1995). Moreover, expression of p45 along with Mafg and Mafk is sufficient to convert mouse 3T3 and human dermal fibroblasts into megakaryocytes (Ono et al. 2012).

The ETS family transcription factor, Fli-1, is an important regulator of the late stages of megakaryocyte differentiation. Germline inactivation of Fli-1 in mice results in embryonic lethality associated with an early block in the differentiation of megakaryocytes (Hart et al. 2000). Moreover, germline mutations in FLI1 have been associated with platelet dysfunction, thrombocytopenia, and bleeding disorders in human patients underscoring the importance of FLI-1 in platelet formation (Daly 2017). Functionally, Fli-1 cooperates with GATA-1 for the activation of genes associated with the terminal differentiation of megakaryocytes. Another ETS family transcription factor, PU.1, is important in the development of platelets, whereby PU.1 repression is needed for the normal development of megakaryocyte-erythroid progenitors (DeKoter et al. 2007; Niswander et al. 2014).

1.4.2 Growth Factor Signaling

Megakaryopoiesis and platelet formation occur within the bone marrow microenvironment. Cytokines and other factors within the microenvironment play an important role in megakaryocyte differentiation and platelet formation. In vitro studies have demonstrated that human interleukin-3 (IL-3), interleukin-6 (IL-6), stem cell factor (SCF), and thrombopoietin (TPO) promote megakaryocyte differentiation and proliferation (Guo et al. 2015). Among these factors, TPO is the primary regulator of MK development and platelet formation (Kaushansky et al. 1995). TPO binds to the MPL receptor that is expressed on megakaryocyte progenitor cells to induce proliferation and differentiation (Gurney et al. 1994). TPO is primarily produced by the liver at constitutive levels and is cleared by MPL receptor in platelets (Emmons et al. 1996).

1.4.3 Microenvironment

Within the bone marrow microenvironment, an important role for the vascular niche in the regulation of megakaryocyte maturation and platelet formation has recently been described. MK association with the sinusoidal vasculature promotes proplatelet formation and platelet release into the circulation (Niswander et al. 2014; Junt et al. 2007; Pitchford et al. 2012). Future studies are needed to further explore the cellular and molecular mechanisms controlling platelet formation in the bone marrow microenvironment.

1.5 Monocyte

Monocytes are produced in the bone marrow and circulate in the blood stream and enter tissues where they differentiate into macrophages and dendritic cells. Both macrophages and dendritic cells contribute to host defense, tissue remodeling, and repair (Gordon and Taylor 2005).

1.5.1 Genetic and Epigenetic Regulation

The development of monocytes from pluripotent HSCs occurs through several myeloid progenitors including the common myeloid progenitor (CMP), granulocyte/macrophage precursor (GMP), and a macrophage/DC precursor (MDP). Recently, a DC-restricted progenitor (CDP) and a monocyte/macrophage-restricted progenitor (cMoP) downstream of the MDP have been described. Monopoiesis occurs in the bone marrow through the coordination of transcription

factor expression. There are a number of transcription factors that coordinate this process.

The ETS transcription factor PU.1 plays an important role in monocyte differentiation. While PU.1 expression inhibits the differentiation of the granulocyte lineage, PU.1 is required for macrophage and dendritic cell development (Dahl et al. 2003). PU.1 is expressed in early myeloid lineage commitment and activates the expression of monocyte-specific factors including IRF8 and KLF4 to support monocyte differentiation (Schonheit et al. 2013; Feinberg et al. 2007).

The interferon-gamma (IFN-g)-regulated transcription factor, IRF8, plays a key role in monocyte differentiation. While IRF8-deficient mice are viable, these mice accumulate GMP, expand neutrophils, and are deficient in macrophages and dendritic cells (recently reviewed in (Terry and Miller 2014)).

The Kruppel-like factor KLF4 plays an important role in monocyte differentiation. KLF4 is expressed in monocyte-restricted progenitors (Feinberg et al. 2007). Overexpression of KLF4 in HSCs promotes monocyte differentiation, whereas KLF4 knockdown inhibits monocyte differentiation (Feinberg et al. 2007). Furthermore, introduction of KLF4 into PU.1 or IRF8-deficient cells is sufficient to rescue monocyte development (Terry and Miller 2014). In support of this notion, KLF4-deficient mice die within a few days after birth. Transplantation of KLF4-deficient fetal liver cells into lethally irradiated mice resulted in a complete loss of circulating monocytes (Alder et al. 2008). Together these studies suggest that KLF4 is an important downstream factor controlling monocyte development.

The nuclear receptor NR4A1 (Nur77) is a transcription factor that has recently been identified in the differentiation of Ly6C$^-$ monocytes. Ly6C$^-$ monocytes are surveillance monocytes present in the circulation that have recently been implicated in controlling metastasis, monitoring endothelial cells, inflammation, and tissue repair (Terry and Miller 2014). Germline inactivation of NR4A1 resulted in a specific depletion of Ly6C$^-$ monocytes. NR4A1 is important in promoting the survival and differentiation of Ly6C$^-$ monocytes (Hanna et al. 2011).

1.5.2 Growth Factor Signaling

There are a number of cytokines and growth factors that promote monocyte differentiation and regulate macrophage and dendritic cell development. The receptor tyrosine kinase CD115 (CSF-1R) is expressed by early myeloid progenitors and is required for monocyte survival, proliferation, and differentiation. Mice that are deficient for CSF-1R lack most monocyte and macrophage populations (Dai et al. 2002). There are two ligands for CSF-1R, CSF-1, and IL-34. Germline inactivation of these factors results in monocyte and macrophages deficiencies (for recent review (Terry and Miller 2014)). During inflammatory conditions, the cytokines interferon-gamma (IFN-gamma) and type I interferon (IFN-1) promote monocyte development. Flt3 ligand (Flt3L) is an important regulator of dendritic cell development. The Flt3 receptor is expressed by dendritic cells throughout development and is maintained on terminally differentiated macrophages (Satpathy et al. 2012). Loss of Flt3L, Flt3, or its key downstream signaling molecule STAT3 results in dendritic cell depletion in mice (McKenna et al. 2000; Waskow et al. 2008; Laouar et al. 2003).

1.6 Neutrophil

Neutrophils represent 50–75% of the total circulating leukocytes, play an important role in inflammation, and are early responders to pathogens including bacteria, fungi, and viruses. They mediate direct antimicrobial activities through the release of enzymes and toxic factors, the generation of reactive oxygen species, and the release of nuclear material into extracellular traps. In addition, neutrophils regulate adaptive and innate immune responses through the release of cytokines and pattern recognition receptors. Granulocyte differentiation occurs in the bone marrow through a series of developmental stages. The myeloblast is a neutrophil and monocyte progenitor that arises from the common myeloid progenitor. After the myeloblast stage, granulopoiesis promotes the differentiation of neutrophils through the formation

of promyelocytes, myelocytes, metamyelocytes, and band cells ultimately leading to polymorphonuclear neutrophils. Neutrophils then exit the bone marrow and undergo terminal maturation in the bloodstream. Neutrophils have a half-life of 4–10 h in circulation and survive in tissues for up to 2 days. Therefore, these cells need to be continuously replenished through the coordinated regulation of growth factor receptor signaling and transcriptional regulation of neutrophil-specific gene programs (for a recent review, see (Ostuni et al. 2016; Barreda et al. 2004)).

1.6.1 Transcription Factors

There are a number of transcription factors that regulate neutrophil development. Early in monocyte/granulocyte lineage commitment, the balance between PU.1 and C/EBPα expression plays an important role in specifying monocyte and neutrophil production. High PU.1 levels favor the development of macrophages and dendritic cells, whereas high levels of C/EBPα promote neutrophil differentiation (for a recent review, see (Ostuni et al. 2016)). Later during terminal neutrophil commitment, the transcription factors C/EBPε, GFI-1, and LEF-1 play important roles in the terminal differentiation of neutrophils.

The CCAAT/enhancer-binding protein-E (C/EBPε) transcription factor promotes the terminal differentiation and maturation of neutrophils. C/EBPε is highly expressed in granulocytes where it is essential for their terminal differentiation. Mice with an inactivating mutation in C/EBPε are viable. However, C/EBPε-deficient mice fail to produce functional neutrophils and eosinophils and succumb to opportunistic infections by 3–5 months of age (Yamanaka et al. 1997). C/EBPε is essential for the expression neutrophil granule proteins including lactoferrin and defensins (Khanna-Gupta et al. 2003; Tsutsumi-Ishii et al. 2000). Interestingly, many of the features observed in in C/EBPε-deficient mice are observed in patients with SGD, a rare autosomal recessive primary immunodeficiency that develops from a neutrophil-specific granule deficiency. Recent studies have identified a variety of mutations within C/EBPε in patients with SGD supporting an important role for C/EBPε in the physiologic and pathophysiologic regulation of neutrophils (Lekstrom-Himes et al. 1999; Gombart et al. 2001; Wada et al. 2015).

The zinc finger transcriptional repressor Gfi-1 plays an essential role in neutrophil differentiation. Genetic inactivation of Gfi-1 in mice results in growth retardation, early lethality, and bacterial infection. Peripheral blood analysis in Gfi-1-deficient mice revealed a specific defect in neutrophils associated with a block in neutrophil differentiation at the myeloblast to promyelocyte transition (Hock et al. 2004). Mechanistically, Gfi-1 is required for the expression of neutrophil-specific genes including MPO, C/EBPε, and elastase (Hock et al. 2004; Person et al. 2003). Clinically, mutations within GFI1 have been found in patients with severe congenital neutropenia (SCN), a rare autosomal recessive disorder characterized by a defect in neutrophil maturation and a risk of life-threatening bacterial and fungal infections (Person et al. 2003).

Another important transcription factor in neutrophil differentiation is the lymphoid enhancer-binding factor (LEF-1). LEF-1 is a transcription factor that is normally expressed in lymphoid cells. However, promoter analysis of ELA2 (protease neutrophil elastase), a gene linked to severe congenital neutropenia, revealed a functional LEF-1 binding site within the ELA2 promoter that cooperates with core-binding factor (CBFalpha) in the activation of ELA2 expression (Li et al. 2004). Clinically, low LEF-1 levels are associated with congenital neutropenia where neutrophils arrest at the promyelocytic stage. Functional studies in early hematopoietic progenitor cells from patients with congenital neutropenia demonstrated that reconstitution of LEF-1 is sufficient to rescue defective myelopoiesis and granulocyte maturation (Skokowa et al. 2006).

1.6.2 Growth Factor Signaling

Neutrophil production is regulated by the expression of granulocyte colony-stimulating factor (G-CSF). G-CSF promotes neutrophil proliferation,

differentiation, and survival through the activation of the G-CSF receptor expressed on neutrophilic progenitors and mature neutrophils. During neutrophil maturation, the expression of G-CSFR increases with mature neutrophils expressing 200–1000 receptors per cell. The G-CSF receptor is a member of the type I cytokine receptor superfamily that binds to G-CSF as a homodimer. The extracellular domain contains an immunoglobulin-like (Ig-like) domain, a cytokine receptor homology (CRH) module that is required for G-CSF binding, and three fibronectin type II domains. The cytoplasmic domain contains Box1 and Box2 domains that have homology to other cytokine receptors and a STAT3 binding site. Intracellular signaling downstream of the G-CSF receptor relies upon cytoplasmic enzymes because the G-CSFR does not have an intrinsic tyrosine kinase activity. G-CSF binding to G-CSFR results in homodimerization of the receptor and the formation of a tetrameric complex containing two ligand and two receptor complexes. G-CSFR activation results in the activation of JAK, STAT, Erk, and Src family kinase signaling that mediates the biologic activities associated with G-CSF signaling in neutrophils (for a recent review, see (Barreda et al. 2004)).

1.6.3 Microenvironment

A variety of cells within the microenvironment regulate neutrophil production under homeostatic and stress conditions. Among these cell types, macrophages play an important in the regulation of steady state and stress granulopoiesis. Under homeostatic conditions, apoptotic neutrophils in the periphery and bone marrow are ingested by resident tissue macrophages. This results in the suppression of proinflammatory cytokine production by macrophages and a reduction of G-CSF production by T cells (Stark et al. 2005). Under stress conditions, bacterial lipopolysaccharide (LPS) IL-1, M-CSF, and other factors stimulate macrophages and monocytes to produce G-CSF (for a review, see (Barreda et al. 2004)). In addition to macrophages, dendritic cells regulate neutrophil homeostasis by regulating the recruitment and survival of neutrophils in peripheral tissues. Conditional depletion of dendritic cells results in increased mobilization of neutrophils from the bone marrow to the liver. Increased neutrophils in the liver of dendritic cell depleted mice were associated with the increased expression of neutrophil-mobilizing cytokines and reduced neutrophil apoptosis in the liver (Jiao et al. 2014). Finally, recent studies have highlighted a role for the microbiota in the regulation of neutrophil aging through the activation of Toll-like receptor and myeloid differentiation factor 88-mediated signaling (Zhang et al. 2015).

1.7 Eosinophil

Eosinophils are granulocytic white blood cells that play an important role in innate and adaptive immunity. Eosinophils differentiate in the bone marrow and exit the bone marrow as terminally differentiated cells. Under homeostatic conditions, eosinophils are found at low levels in the blood (<5% of leukocytes) and primarily reside in tissues such as the gut mucosa; there they maintain homeostasis with resident microbiota (Davoine and Lacy 2014). Eosinophils are not capable of proliferating and have high rates of spontaneous apoptosis. It is estimated that their lifespan is 2–5 days within resident tissues. Therefore, the production of eosinophils is a tightly regulated process that is needed to maintain eosinophil levels within tissues while maintaining low levels of these sensitive cells within the blood. Eosinophils develop in the bone marrow over 3–4 days from pluripotent stem cells and granulocyte-monocyte progenitors to an eosinophil lineage-committed progenitor (EoP) (Doyle et al. 2013). There are number of genetic, epigenetic, and microenvironmental factors that control eosinophil differentiation and survival in the bone marrow that will be discussed below.

1.7.1 Genetic

Eosinophil development results from carefully coordinated activation of transcription factors. The transcription factors GATA-1, PU.1, C/

EBPa, C/EBPe, IRF8, and XBP-1 are expressed by eosinophils and are required for their specification and differentiation (Yu et al. 2002; McKercher et al. 1999; Zhang et al. 1997; Tamura et al. 2015; Bettigole et al. 2015). GATA-1 is particularly important in the specification of the eosinophil lineage. Targeted deletion of the high-affinity GATA-1 binding site in the GATA-1 promoter results in eosinophil lineage depletion in mice (Yu et al. 2002). Moreover, a high-affinity double-GATA site is present within the regulatory elements of eosinophil-specific genes including the eotaxin receptor CC chemokine receptor 3 (CCR3), MBP, and the IL-5 receptor alpha (IL-5Rα) genes (Du et al. 2002; Kim et al. 2010; Yamaguchi et al. 1998).

Recent studies have demonstrated a key role for the transcription factor XBP-1 in eosinophil differentiation. XBP-1 is a transcription factor that mediates the cellular response to endoplasmic reticulum (ER) stress by activating the expression of genes that encode proteins that regulate protein folding, maturation, and degradation within the ER (Lee et al. 2003). During differentiation, eosinophils activate the expression of XBP1 to enhance the posttranslational maturation of key granule proteins, which are thought to allow for the progression of the eosinophil transcriptional developmental program (Bettigole et al. 2015).

Eosinophils are unique among granulocytes in that they must safely store a variety of cytotoxic proteins including eosinophil major basic proteins (MBP-1, MBP-2), eosinophil-associated ribonucleases, and eosinophil peroxidase (EPX) (Lee et al. 2012). The survival of eosinophils during differentiation is also dependent upon granule formation. Gene-targeting studies in mice have demonstrated that granule protein expression, specifically MBP-1 and EPX, is required for eosinophilopoiesis suggesting that granule protein expression and/or formation is not a passive consequence of differentiation but also plays an important role in the differentiation process (Doyle et al. 2013).

Studies investigating gene expression and chromatin changes during eosinophil development have been limited, likely because of the rarity of the cell population under homeostatic conditions. A recent study utilized chromatin immunoprecipitation coupled with massively parallel sequencing (ChIP-seq) and RNA sequencing (RNA-seq) to identify transcription factors and genetic regulatory elements that are active during homeostatic eosinophil production in the bone marrow (Bouffi et al. 2015). This study found that genes that are induced eosinophil lineage-committed cells (EoPs) were poised with active chromatin marks in GMPs. In contrast genes that are highly and specifically expressed during eosinophil maturation were not marked with open chromatin at early stages, suggesting that a subset of genes associated with Eos effector functions may be poised for expression early in lineage commitment (Bouffi et al. 2015).

1.7.2 Growth Factor Signaling

There are three cytokines that are important in eosinophil development including IL-5, IL-3, and GM-CSF. IL-5 is particularly important both eosinophil differentiation and release from the bone marrow into the circulation. Overproduction of IL-5 in transgenic mice leads to eosinophilia, whereas IL-5 or the IL-5 receptor α chain (IL-5Rα)-deficient mice fail to develop parasite-induced eosinophilia (Kopf et al. 1996; Tominaga et al. 1991; Dent et al. 1990; Yoshida et al. 1996). Clinically, overproduction of IL-5 is associated with eosinophilia (Agache et al. 2016; Van Gool et al. 2014; Pavord et al. 2012).

Interleukin-5, IL-3, and GM-CSF signal through the common beta-receptor chain (βc) to provide proliferative and differentiation signals to eosinophil progenitors. Mice lacking the common beta-receptor have a reduced number of peripheral eosinophils under homeostatic conditions highlighting the importance of IL-5/IL-3/GM-CSF signaling in eosinophil production (Nishinakamura et al. 1996). The IL-5 receptor complex is comprised of an IL-5 homodimer, two IL-5Ralpha subunits, and one beta-receptor chain (Broughton et al. 2015). IL-5 receptor signaling activates Lyn, Syk, and JAK2 and signals through the Ras-MAPK, JAK-STAT, and PI3K/

AKT pathways to promote eosinophil proliferation, differentiation, and survival (Adachi and Alam 1998).

1.7.3 Microenvironment

The primary cellular source of serum IL-5 during homeostatic conditions is maintained by long-lived type II innate lymphoid cells resident in peripheral tissues (Nussbaum et al. 2013). Within the bone marrow niche, eosinophils are localized with plasma cells. While eosinophils are important in providing plasma cells with survival factors, the role of plasma cells on eosinophil function remains poorly understood (Chu et al. 2011).

1.8 Basophil and Mast Cells

Basophils and mast cells are granulocytes that play an important role in mediating allergy and parasite immune responses. Both cell types express high-affinity immunoglobulin E (IgE) receptors on their cell surface that leads to the release of cytokines and chemical mediators upon antigen stimulation. Basophils and mast cells develop in the bone marrow from common myeloid progenitors (CMP) and granulocyte-monocyte progenitors (CMP). Current models suggest that bipotential granulocyte progenitors differentiate into pre-basophil and mast cell progenitors (pre-BMPs) and then to basophil or mast cell progenitors. While basophil maturation occurs in the bone marrow, mast maturation occurs primarily within peripheral tissues. Basophil and mast cell differentiation utilizes cues from the microenvironment to coordinate transcription factor gene expression that is necessary for lineage differentiation (for a recent review, see (Sasaki et al. 2016)).

1.8.1 Transcription Factors

There are multiple transcription factors that coordinate basophil and mast cell differentiation in the bone marrow including IRF8, C/EBPa, GATA2, microphthalmia-associated transcription factor MITF, STAT5, and RUNX1.

The interferon regulatory factor-8 (IRF-8) is a transcription factor required for the development of several myeloid lineages described above. Recent studies have identified an important role for IRF-8 in the differentiation of basophil and mast cells. Irf8 inactivation in mice led to a severe defect in peripheral basophil counts that was associated with a block in GMP to pre-BMP differentiation in the bone marrow. While tissue resident mast cells were maintained in Irf8-deficient mice, GMP cells from these mice failed to differentiate into mast cells suggesting an important role for Irf8 in mast cell and basophil production in vivo (Sasaki et al. 2015). Future studies are needed to elucidate the mechanisms by which IRF8 controls mast and basophil differentiation.

Recent studies have demonstrated that the coordinate regulation of STAT5, C/EBPa, and MITF controls the differentiation and specification of basophil and mast cell progenitors in the bone marrow. The transcription factor C/EBPa plays an important role in promoting the differentiation of pre-BMPs into basophils. Cebpa mRNA is upregulated during the GMP to pre-BMP transition through STAT5, an essential signaling molecule for basophils and mast cells as conditional inactivation of Cebpa in adult mice resulted in a block in pre-BMP development in the bone marrow (Sasaki et al. 2016; Qi et al. 2013). At the molecular level, C/EBPa promotes basophil differentiation and identity by actively repressing genes that are highly expressed within the mast cell lineage. In particular, C/EBPa directly binds to and suppresses the activation of Mitf, a transcription factor that is required for mast cell differentiation in vivo (Sasaki et al. 2016; Qi et al. 2013).

As described above, GATA2 is an important factor in the early stages of hematopoiesis. Recent studies have revealed an important role for GATA2 in mast cell and basophil differentiation. Gene expression profiling of factors that are highly expressed in the GMP to pre-BMP transition identified GATA2 as a factor highly expressed in pre-BMPs. Conditional inactivation of GATA2 in pre-BMPs leads to a block in