Perspective

Leukemia and hematopoietic stem cells

Balancing proliferation and quiescence

Craig D. Jude,1,† Justin J. Gaudet,2,† Nancy A. Speck2,3 and Patricia Ernst1,3,*

Departments of 1Genetics and 2Biochemistry; 3Norris Cotton Cancer Center; Dartmouth Medical School; Hanover, New Hampshire USA

†These authors contributed equally to this work.

Abbreviations: HSC, hematopoietic stem cell; LT-HSC, long-term engrafting hematopoietic stem cell; ST-HSC, short-term engrafting hematopoietic stem cell; MPP, multipotent progenitor; CDK, cyclin dependent kinase; LSK, lineage negative, Sca-1 positive, c-Kit positive; polyI:C, polyinosinic polycytidylic acid; Met, methionine; Val, valine; S/G2/M, S phase/gap 2/mitosis; AML, acute myelogenous leukemia; PI3K, phosphatidylinositol 3-kinase

Key words: HSCs, cell cycle, quiescence, leukemia, transcription, chromatin

Chromosomal translocations that disrupt transcriptional regulators are frequently involved in the etiology of leukemia. To gain an understanding of the normal and pathologic roles of these transcriptional regulators, both gain- and loss-of-function mutations have been examined in the context of steady-state hematopoiesis. These studies have identified a remarkable number of genes whose loss-of-function phenotype includes a perturbation of hematopoietic stem cell (HSC) proliferation. As more of these models are generated and analyzed using commonly available tools, the regulatory pathways that control HSC quiescence and proliferation are becoming clearer. An emerging theme is that leukemia-associated transcriptional regulators coordinate the balance of proliferation and quiescence within the HSC pool by modulating the number and frequency of cells transiting the cell cycle. Uncoupling proliferation from differentiation by the aberrant generation of chimeric oncogenes that retain some, but not all of the attributes of the original transcription factor is likely to be an important step during leukemogenesis.

Introduction

One approach to understanding the genetic pathways deregulated in leukemia is to study the normal functions of the proto-oncogenes involved in chromosomal translocations that typify particular leukemia subsets. In contrast to the proto-oncogenes and tumor suppressors made familiar by classic solid tumor studies,1,2 the players in leukemia are numerous and less familiar. This may be due to the fact that in contrast to most solid tumors, leukemia arises in cells in which proliferation and differentiation are ongoing processes. Leukemia has been described as a disease in which a block in differentiation plays an important role in the transformation process.3 This observation may simply result from the fact that it is common for the coordination between cell identity and proliferation to be performed by individual transcriptional regulators. Studies characterizing the roles of individual cell cycle regulators in adult hematopoiesis have demonstrated that loss of core cell cycle components (including cyclin-dependent kinases [CDKs] and their inhibitors) have distinct effects on stem and progenitor homeostasis and function as reviewed by Walkey et al.4 These studies provide an essential point of reference for the work reviewed here, but are not discussed in detail in this review.

Here we review a selection of recent studies that characterize the function of specific genes in hematopoiesis, most of which have been implicated in leukemia by their identification as frequent targets of chromosomal translocation. However, we also discuss several genes whose involvement in leukemia was implicated by other experimental evidence. The genes discussed below are divided broadly into three basic groups (Fig. 1): those whose mutation results in (1) an increase in phenotypically defined HSCs with an equivalent increase in HSC function (“Homeostatic Negative Regulators”), (2) an increase in phenotypically defined HSCs accompanied by a decrease in HSC function (“Homeostatic Balancers”), and (3) a concomitant reduction in phenotypically defined HSC number and function (“Homeostatic Positive Regulators”).

Homeostatic Negative Regulators

This first group of genes include those that when mutated, result in an increased HSC pool size accompanied by an increase in HSC function (Fig. 1 and Table 1). The very existence of this category illustrates that active processes are in place to suppress HSC numbers under steady-state conditions.

Loss-of-function mutations of the proto-oncogene c-Myb demonstrate its essential role during hematopoietic development.5,7 In addition, a co-activator recruited by c-Myb, p300, is required for differentiation of multiple hematopoietic cell types.8,9 However, HSCs in which c-Myb has selectively lost the ability to interact with
p300 exhibit augmented steady-state HSCs and enhanced HSC function. This selective disruption is characteristic of a particular mutant allele of c-Myb (Met303 → Val, isolated by chemically induced mutagenesis). Competitive transplantation assays using bone marrow from these homozygous mutant animals revealed a 5–10 fold elevation in HSC frequency, which was consistent with the increase in HSC pool size as measured by cell surface phenotype. In this and other studies described in the following sections, the lineage-negative, Sca-1 positive and c-Kit positive (LSK) population serves as a surrogate measure of the HSC pool, as this population is highly enriched in HSCs. Analysis of LSK cells from the Met303 → Val c-Myb mutant revealed roughly twice as many of these cells in the S/G2/M phase of the cell cycle relative to wild-type. Despite the increase in proliferation within the LSK pool, the c-Myb mutant had functionally overabundant HSCs as revealed through 3 rounds of serial transplantation, suggesting that the increased number of cycling cells has no negative consequences on HSC function. This example, like that of Hoxb4 overexpression, also illustrates that an increase in HSC pool size can be maintained without necessarily resulting in leukemia.

An interesting contrast is exemplified by the Ets-domain protein MEF/Elf-4 which has been implicated in M2/M3 acute myelogenous leukemia (AML). Constitutive loss of Elf4 results in an increase in the phenotypic long-term (LT)-HSC (here defined as LSK/CD34-/Flt-3-) with some key differences with respect to proliferation. Elf4 knockout cells out-compete wild-type cells in transplantation assays and knockout animals recover faster than wild-type mice from myeloablation. However, in contrast to the c-Myb mutant described above, steady-state or cytokine-stimulated Elf4-null HSCs exhibit a reduction in S/G2/M cells, reduced BrdU uptake, and a resistance to enter the cell cycle upon cytokine stimulation: Despite this cell cycle perturbation within the HSC pool, Elf4 knockout animals apparently maintain normal bone marrow cellularity. These examples illustrate that the loss of transcriptional regulators or their interaction with co-activators can lead to coordinately enhanced HSC number and function. Importantly, enhanced HSC pool size and function can be achieved in conjunction with enhanced or reduced proliferation within the HSC pool, suggesting there are multiple mechanisms by which HSC numbers are normally suppressed.

**Homeostatic Balancers**

Considering that the ability to maintain a quiescent state is an important characteristic of HSCs, a more intuitive response to deregulated proliferation might be the loss of HSC function, which is a common phenotype in many of the knockout models analyzed to date. The Gfi1 gene knockout was one of the first targeted mutants to exhibit the combination of an increase in cycling HSCs at the expense of HSC function. Gfi1 was identified as a proto-oncogene activated by proviral insertion in a mouse model of T cell lymphoma. Although bone marrow HSC numbers were slightly increased in one study and decreased in another, both Gfi1 knockout models exhibited an increase in cycling cells within the HSC pool, a large decrease in expression of the cyclin-dependent kinase inhibitor p21, and profoundly reduced HSC function in transplantation experiments. Thus, under normal homeostasis, Gfi1 is thought to suppress the proliferation of HSCs thereby preventing their depletion.

The excision of the tumor suppressor gene, JunB, results in some phenotypes in common with the Gfi1 knockout described above. Due to the embryonic lethality, JunB excision was achieved in HSCs using the polyinositide-polycytidylic acid (polyI:C) inducible Mx1-Cre transgene. Upon JunB loss, an expanded HSC pool was observed, specifically in the LT-HSC population (here defined as LSK/Flt-3-/Thy-1low). This expansion is accompanied by a greater percentage of LT-HSCs in S/G2/M and reduced engraftment. These animals eventually succumb to a myeloproliferative disorder that is initiated in the HSC pool, demonstrating that the accumulated HSCs are susceptible to additional events that result in leukemia.

MYC was discovered as a proto-oncogene in lymphoma, and has over the intervening two decades been implicated in a wide variety of solid tumors and leukemias. The acute loss of Myc in adult bone marrow results in the transient accumulation of HSCs as measured by phenotype (LSK/Flt3^+), then a progressive loss of bone marrow cells which is maximal at 8 weeks after Myc deletion. Perinatal deletion of Myc also using the Mx1-Cre system results in the accumulation of lineage negative, Sca-1 positive, c-Kit negative cells, which are proposed to reflect an aberrant, senescent primitive progenitor. Myc-deficient HSCs produced by either perinatal or adult excision fail to engraf in a competitive or non-competitive setting, demonstrating that the accumulated cells are functionally
HSC pathways controlling proliferation and quiescence

The accumulation of phenotypic HSCs appears to result from a combination of a slight proliferative increase within the HSC pool (possibly due to N-Myc substitution at Myc targets) and mis-expression of cell surface molecules that may retain HSCs within the bone marrow microenvironment.

**RUNX1/AML1** is disrupted by chromosomal translocation in a majority of childhood leukemias, often producing a fusion protein that is thought to act by a dominant-interfering mechanism. Excision of *Runx1* in adult bone marrow, again using the *Mx1-Cre* transgene, caused the stem and early progenitor fraction of cells to expand by as much as 3 fold. Interestingly, LT-HSCs isolated from these mice exhibit a proliferation-associated gene expression signature, similar to that of the short-term HSC/multipotent progenitor population (ST-HSC/MPP) despite retaining the LT-HSC cell surface phenotype.

**Disruption of several important developmental signaling pathways can also result in similar phenotypes as described for the transcriptional regulator knockout models described above.** For example, acute activation of Wnt signaling, accomplished by excising exons encoding negative regulatory sequences of β-catenin, using the *Mx1-Cre* model, resulted in a transient increase in the number of LSK cells and an increase in the proportion that are in cycle. These cells also failed to sustain hematopoiesis in a transplant setting or in the original *Mx1-Cre* animal, which succumbs to bone marrow failure within several weeks. The rapid lethality may result from both the depletion of HSC function and a block in the development of multiple progenitors.

Constitutive activation of the Hedgehog (Hh) signaling pathway in *Patched (Ptc)* heterozygotes similarly results in the steady-state accumulation of phenotypically-defined HSCs and an increase in the proportion of cycling cells within this population. In contrast to activation of the Wnt pathway described above, enhanced short-term engraftment in primary recipients is observed using *Ptc*; bone

---

Table 1  Mutant classification, cell cycle phenotype and impact on HSC reconstitution of hematopoiesis

<table>
<thead>
<tr>
<th>Category</th>
<th>Gene name</th>
<th>KO system</th>
<th>Target cell immunophenotype</th>
<th>Cell cycle defect</th>
<th>Engraftment capacity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homeostatic negative</td>
<td>c-Myb</td>
<td>constitutive,</td>
<td>IL-7Rα⁺, L/S⁺, K⁺</td>
<td>cells shift from G0/S0/G1 to G0/G1/M</td>
<td>enhanced serial/competitive engraftment</td>
<td>10</td>
</tr>
<tr>
<td>regulators (HNRs)</td>
<td></td>
<td>hypomorphic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meis1</td>
<td>constitutive</td>
<td>L/S⁺, K⁺, Flt3⁺, CD34⁺</td>
<td>cells shift from G0/S0/G1 to G0/G1/M</td>
<td>enhanced secondary competitive engraftment</td>
<td>15</td>
</tr>
<tr>
<td>Homeostatic Balancers</td>
<td>Gfi1</td>
<td>constitutive</td>
<td>L/S⁺, K⁺, Flt3⁺</td>
<td>cells shift from G0/S0/G1 to G0/G1/M</td>
<td>reduced serial/competitive engraftment</td>
<td>16</td>
</tr>
<tr>
<td>(HBs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JunB</td>
<td>inducible*</td>
<td>L/S⁺, K⁺, Flt3⁺</td>
<td>cells shift from G0/S0/G1 to G0/G1/M</td>
<td>defective long-term/competitive engraftment</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>c-Myc</td>
<td>inducible*</td>
<td>L/S⁺, K⁺, Flt3⁺</td>
<td>none apparent</td>
<td>reduced HSC differentiation, progenitor</td>
<td>20,21</td>
</tr>
<tr>
<td></td>
<td>Cdk42</td>
<td>inducible*</td>
<td>L/S⁺, K⁺, CD34⁺</td>
<td>increased # of BrdU⁺ cells</td>
<td>defective long-term engraftment</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Runx1</td>
<td>inducible*</td>
<td>IL-7Rα⁺, L/S⁺, K⁺</td>
<td>ND⁺</td>
<td>defective long-term engraftment</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Runx1</td>
<td>inducible*</td>
<td>CD34⁺, L/S⁺, K⁺</td>
<td>ND⁺</td>
<td>defective long-term/competitive engraftment</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Pten</td>
<td>inducible*</td>
<td>L/S⁺, K⁺, Flt3⁺</td>
<td>ND⁺</td>
<td>defective long-term/competitive engraftment</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>FoxO1/03/04</td>
<td>inducible*</td>
<td>L/S⁺, K⁺, Flt3⁺</td>
<td>ND⁺</td>
<td>defective long-term/competitive engraftment</td>
<td>31</td>
</tr>
<tr>
<td>Homeostatic positive</td>
<td>Bm1</td>
<td>constitutive</td>
<td>L/S⁺, K⁺, CD34⁺, Fetal Liver E14</td>
<td>none apparent</td>
<td>defective long-term engraftment</td>
<td>45</td>
</tr>
<tr>
<td>regulators (HPRs)</td>
<td>M33</td>
<td>constitutive</td>
<td>fetal Liver E14.5</td>
<td>ND⁺</td>
<td>normal engraftment</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Mesi1</td>
<td>constitutive</td>
<td>whole bone marrow</td>
<td>ND⁺</td>
<td>enhanced engraftment</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Mesi1</td>
<td>constitutive</td>
<td>fetal Liver E14.5</td>
<td>ND⁺</td>
<td>defective long-term engraftment</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Raeo1</td>
<td>constitutive</td>
<td>constitutive</td>
<td>none apparent</td>
<td>defective long-term engraftment</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Mili</td>
<td>inducible*</td>
<td>L/S⁺, K⁺, CD48⁺</td>
<td>cells shift from G0 to G0/S0/G1/M</td>
<td>defective long-term/competitive repopulation</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Mili</td>
<td>Vav-Cre</td>
<td>fetal Liver E13.5</td>
<td>cells shift from G0 to G0/S0/G1/M</td>
<td>defective long-term/competitive repopulation</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>MOZ</td>
<td>constitutive</td>
<td>fetal Liver E14</td>
<td>ND⁺</td>
<td>defective long-term/competitive repopulation</td>
<td>59</td>
</tr>
</tbody>
</table>

†Mx1-Cre unless otherwise stated; §No Data; The genes categorized into the 3 groups described in this review are listed. The system column indicates whether a germline knockout or a conditional, inducible knockout was analyzed. The descriptive columns summarize the definition of steady state HSC populations analyzed, the cell cycle perturbations within this phenotype, and the functional capacity of these cells. The reference number is indicated in the last column.
marrow cells. However, HSC activity upon secondary transplantation is reduced ~3 fold, indicating the functional exhaustion of the HSC pool in this mutant.36

The disruption of phospho-inositol-3 kinase (PI3K) signaling pathways through PTEN or FoxO1/3/4 deletion using the Mxl-Cre transgene exhibits features in common with those models described above. In both the PTEN and FoxO1/3/4 conditional knockout models, acute loss of gene function is associated with a transient increase in phenotypically-defined HSCs, accompanied by an increase in the proportion of HSCs that are cycling.37-40 Both the ability of these cells to persist in the bone marrow of chimeras and to engraft secondary recipients is highly compromised. A slight increase in apoptosis appears to play a role in the homeostasis of HSCs in the FoxO1/3/4 knockout but not in the PTEN knockout.37 Ultimately, both animals acquire a myeloproliferative syndrome or leukemia, but the defects in HSC proliferation and function are temporally and clonally separable from these diseases.

Lastly, an Mxl-Cre mediated knockout of cdc42, a Rho family GTPase, similarly results in a transient increase in ST-HSCs (CD34+ LSK immunophenotype), coupled with a rapid shift from quiescence to a proliferative state within the LSK pool overall.41 In this particular model, HSC mobilization (migration out of the bone marrow) to the blood and peripheral organs is substantial, although it is also observed to a lesser extent in the FoxO1/3/4 knockout.42,43 Similar to the other examples, Cdc42-deficient cells exhibit a severe reduction in their ability to engraft recipient animals.

This group of mouse models collectively illustrate that it is common for gene disruptions (JunB, Runx-1, c-Myc, FoxO1/3/4, cdc42) or mutations that activate pathways (Wnt, Hh and PI3K) to promote an accumulation of HSCs at the expense of the long-term preservation of HSC functions. This relationship suggests that multiple independent pathways are actively restricting proliferation within the stem cell pool, and that there exist signals in the environment to promote HSC proliferation when the above pathways are disrupted. The frequent co-occurrence of enhanced proliferation and reduced function within the HSC pool is consistent with studies illustrating that these processes are mechanistically linked.44,45

**Homeostatic Positive Regulators**

This category of genes is required for HSC homeostasis as demonstrated by a continual decline of HSC number and function in loss-of-function models (Fig. 1). Within this category are the Polycomb (PcG) and trithorax (trxG) family genes (Table 1). These gene families were initially identified genetically in fruit flies and demonstrated by a continual decline of HSC number and function in these models. Although the reduced expression of Mll in this model or in a conditional knockout of Mll or Myst3, like Myl, positively regulates Hexo9.55 In addition, the closely related MOF protein has been isolated as part of the MLL multi-protein complex.58 Fetal liver cells from Myst3-/- animals lack both short-term and long-term engraftment potential and exhibit a reduction in phenotypically defined HSCs. 59,60 Although the reduced expression of Hexo genes (amongst others) indicate that Mll and Myst3 may co-regulate shared targets, the distinct phenotypes suggest that this overlap is not extensive.60 Alterations in proliferation within the fetal HSC pool were not reported, but it will be interesting to determine whether proliferation in this model or in a conditional knockout of Myst3 are affected as described above.

**Concluding Remarks**

The genes reviewed here are divided into three categories based on the dynamic perturbation of the HSC pool, as defined both functionally and phenotypically. Many of the gene manipulations discussed here result in embryonic lethality, hence the use of inducible, tissue-selective knockout models has been essential to elucidate functions in adult HSCs. Due to the frequent use of the Mxl-Cre transgenic model, a unique opportunity for direct comparisons exists. Although the categorization presented here may represent an over-simplification in that many of the genes within a category may impinge on
very different pathways to maintain a proper balance of HSCs, it is informative to appreciate shared and distinct features within these groups (Table 1). These comparisons should inform further studies aimed at identifying distinct pathways required to maintain the HSC pool in vivo. Beyond these initial reports, several groups have begun to assess compound mutants in which cell cycle regulatory mutations are combined with mutants described in this review.61,62 Such analyses will establish epistatic relationships among the leukemia-associated transcriptional regulators, signaling pathways, and core cell cycle machinery and hence help define the redundant and unique mechanisms that govern the regulation of HSC proliferation and the decision to self-renew or differentiate.

Due to the dynamic and flexible nature of hematopoiesis, the effects of gene knockouts (either constitutive or conditional) can be complicated by several factors. First, the loss of a given gene product simultaneously at several stages of differentiation can produce an accumulation of particular populations by lengthening the time to differentiate, increasing the production of the input to a particular progenitor pool, or blocking a subsequent differentiation step. This is discussed in particular in the analyses of Runx1 and β-catenin-activated mutations, where an independent block in the development of lymphoid and megakaryocytic, or erythroid, myeloid and lymphoid lineages, respectively, is clearly demonstrated.26,31,33 In addition to performing kinetic analyses of populations after Cre-mediated excision in the inducible knockout models, an informative approach has been to perform analyses of the flux of cycling cells through stem and progenitor pools.25 Second, an important aspect of these knockout studies is the relationship between proliferation and migration out of the bone marrow niche through the circulation.53 Although the extent to which normal HSCs leave the bone marrow during normal steady state hematopoiesis is controversial,64,65 it is clear that a number of perturbations, including agents that produce cytopenia, can increase the rate at which HSCs flux through the circulation and can shift the G0/G1 ratio within the HSC pool. Thus it is important to determine whether these proliferation phenotypes are cell-intrinsic and whether the primary defect is only in the regulation of quiescence, or in aspects of niche retention, which then influence cell proliferation. Future studies will clarify whether the disruption of particular pathways reveals an obligate linkage between bone marrow retention and HSC quiescence, or whether some transcription factor knockouts will reveal an intrinsic control of HSC quiescence.

In conclusion, the detailed analyses of the roles of leukemia-associated transcriptional regulators in adult hematopoiesis has revealed that a common function for these proteins is to maintain the appropriate balance of proliferation and quiescence within the HSC pool. The manipulation of particular signaling pathways and core cell cycle machinery components demonstrates that this field is well on its way toward defining a network of genes whose function is essential to maintain HSC function and that this information will be critical for understanding and interfering with the regulation of leukemogenesis.

Acknowledgements

We extend apologies to our colleagues whose work was not discussed due to space constraints or unintentional omission on the part of the authors. This work was supported by NIH grants DK067119, HL090036 and a NCRR COBRE award (P.E.), AI051427 (N.S.) and T32 AR07576 (J.G.). We are grateful to members of our laboratories for discussion and to R. Mako Saito and David Traver for critical review.

References


