[Cell Cycle 7:5, 1-6; 1 March 2008]; ©2008 Landes Bioscience

Perspective

Leukemia and hematopoietic stem cells

Balancing proliferation and quiescence

Craig D. Jude,[1,†] Justin J. Gaudet,[2,†] Nancy A. Speck[2,3] and Patricia Ernst[1,3,*]

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

[†]These authors contributed equally to this work.

This manuscript has been published online, prior to printing.Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

©2008 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

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/G_2/M, S phase/gap 2/mitosis; AML, acute myelogenous leukemia; PcG, polycomb group; TrG, trithorax group; 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

*Correspondence to: Patricia Ernst; Departments of Genetics; Norris Cotton Cancer Center; Dartmouth Medical School; Hanover, New Hampshire 03755 USA; Tel.: 603.650.1134; Fax: 603.650.1188; Email: patricia.ernst@dartmouth.edu

Submitted: 12/21/07; Accepted: 01/01/08

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/article/5549

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).[10] 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.[11] Analysis of LSK cells from the Met303→Val *c-Myb* mutant revealed roughly twice as many of these cells in the S/G_2/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.[10] This example, like that of *Hoxb4* overexpression,[12] also illustrates that an increase in HSC pool size can be maintained without necessarily resulting in leukemia.

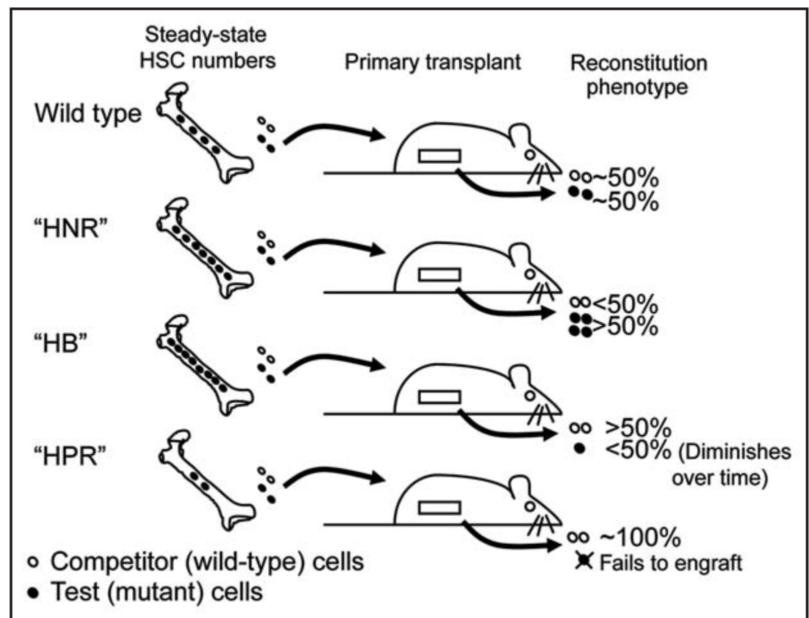

Figure 1. Three basic categories of HSC-specific phenotypes are observed in the knockout animals discussed in this review. Shown are the three categories discussed in the text, their steady-state HSC pool size (circles in the bone icon), and the outcome of primary transplants (indicated on the right for each category). In the first example, wild-type bone marrow cells engraft primary recipients proportional to the number of input cells, indicated by the constant ratio of input cells (left of the mouse) to donor derived cells in the primary transplant recipient (right of the mouse). HNR, homeostatic negative regulators, HB, homeostatic balancer, HPR, homeostatic positive regulator. The closed circles indicate cells from mutant animals and the open circles indicate wild-type competitor bone marrow cells.

An interesting contrast is exemplified by the Ets-domain protein MEF/Elf-4 which has been implicated in M2/M3 acute myelogenous leukemia (AML).[13,14] 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.[15] However, in contrast to the *c-Myb* mutant described above, steady-state or cytokine-stimulated *Elf4*-null HSCs exhibit a reduction in S/G_2/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.[16,17] *Gfi1* was identified as a proto-oncogene activated by proviral insertion in a mouse model of T cell lymphoma.[18] Although bone marrow HSC numbers were slightly increased in one study[16] and decreased in another,[17] 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.[19] Upon JunB loss, an expanded HSC pool was observed, specifically in the LT-HSC population (here defined as LSK/Flt-3-/Thy-1^{low}). This expansion is accompanied by a greater percentage of LT-HSCs in S/G_2/M and reduced engraftment.[20,21] 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.[21]

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.[22,23] 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.[24] *Myc*-deficient HSCs produced by either perinatal or adult excision fail to engraft in a competitive or non-competitive setting, demonstrating that the accumulated cells are functionally

©2008 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

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

Category	Gene name	KO system	Target cell immunophenotype	Cell cycle defect	Engraftment capacity	Ref.
Homeostatic negative regulators (HNRs)	*c-Myb*	constitutive, hypomorphic	IL-7Rα$^-$ L$^-$S$^+$K$^+$	cells shift from G_0/G_1 to $S/G_2/M$	enhanced serial/competitive engraftment	10
	Mef/Elf4	constitutive	L$^-$S$^+$K$^+$ Flt3$^-$ CD34$^-$	cells shift from $S/G_2/M$ to G_0/G_1	enhanced secondary competitive engraftment	15
Homeostatic Balancers (HBs)	*Gfi1*	constitutive	L$^-$S$^+$K$^+$ Flt3$^-$	cells shift from G_0/G_1 to $S/G_2/M$	reduced serial/competitive engraftment	16
	Gfi1	constitutive	L$^-$S$^+$K$^+$ Flt3$^-$, L$^-$S$^+$K$^+$ CD34$^-$	cells shift from G_0/G_1 to $S/G_2/M$	defective long-term/competitive engraftment	17
	JunB	inducible[†]	L$^-$S$^+$K$^+$ Flt3$^-$	cells shift from G_0/G_1 to $S/G_2/M$	reduced	20,21
	c-Myc	inducible[†]	L$^-$S$^+$K$^+$ Flt3$^-$	none apparent	defective HSC differentiation, progenitor expansion	22
	c-Myc	inducible[†]	L$^-$S$^+$K$^+$, L$^-$S$^+$K$^-$	increased # of BrdU+ cells	defective long-term engraftment	24
	Cdc42	inducible[†]	L$^-$S$^+$K$^+$ CD34$^-$	increased # of BrdU+ cells	defective long-term engraftment	41
	Runx1	inducible[†]	IL-7Rα$^-$ L$^-$S^{hi}K^{hi}	ND§	defective long-term competitive/noncompetitive engraftment	29
	Runx1	inducible[†]	CD34$^-$ L$^-$S^{hi}K^{hi}	ND§	defective lymphoid engraftment	30
	Runx1	inducible[†]	L$^-$S$^+$K$^+$	ND§	ND§	31
	Pten	inducible[†]	L$^-$S$^+$K$^+$ Flt3$^-$ CD48$^-$	cells shift from G_0 to $G_1/S/G_2/M$	short-term OK defective long-term/competitive engraftment	40
	Pten	inducible[†]	L$^-$S$^+$K$^+$ Flt3$^-$	cells shift from G_0/G_1 to $S/G_2/M$	defective long-term/competitive engraftment	42
	FoxO1/O3/O4	inducible[†]	L$^-$S$^+$K$^+$ Flt3$^-$	cells shift from G_0 to G_1 and G_1 to $S/G_2/M$	enhanced short-term, defective long-term competitive/noncompetitive engraftment	37
Homeostatic positive regulators (HPRs)	*Bmi1*	constitutive	L$^-$S$^+$K$^+$ CD34$^-$, Fetal Liver E14	none apparent	defective long-term engratment	45
	M33	constitutive	fetal Liver E14.5	ND§	normal engraftment	45
	Mel18	constitutive	whole bone marrow	ND§	enhanced engraftment	45
	Mel18	constitutive	fetal Liver E14.5	ND§	defective competitive engraftment	49
	Rae28	constitutive	constitutive	none apparent	defective long-term engraftment	50
	Mll	inducible[†]	L$^-$S$^+$K$^+$ CD48$^-$	cells shift from G_0 to G_1 and G_1 to $S/G_2/M$	defective longterm/competitive repopulation	52
	Mll	Vav-Cre	fetal Liver E13.5 L$^-$S$^+$K^{hi}	cells shift from G_0 to $G_1/S/G_2/M$	defective longterm/competitive repopulation	53
	MOZ	constitutive	fetal Liver E14.5	ND§	defective longterm/competitive repopulation	59

[†]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.

©2008 LANDES BIOSCIEN

defective.[24,25] 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[24,26]) and mis-expression of cell surface molecules that may retain HSCs within the bone marrow microenvironment.[24,25]

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.[27] 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.[28-31] 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.[28,32] As with the examples above, *Runx1*-deficient HSCs exhibited reduced repopulating activity in both competitive and noncompetitive transplant settings.[29] Furthermore, transplant recipients of *Runx1*-null bone marrow cells experienced a progressive loss of *Runx1*-deficient cells in peripheral blood.[29] Whereas the decline of peripheral white blood cells in *Myc*-deficient mice was accompanied by a progressive decrease in bone marrow cellularity, the cell number in *Runx1*-deficient bone marrow actually increased by a factor of 2, likely due to the subtle, but persistent myeloproliferation that follows conditional ablation of *Runx1* with *Mx1-Cre*.[29] In summary, the examples discussed above exhibit an early reduction of quiescent cells within highly purified HSC populations, demonstrating that these transcriptional regulators act within HSCs to affect the appropriate balance of cycling versus quiescent cells.

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[19,33] 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.[34,35] 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.[34] The rapid lethality may result from both the depletion of HSC function and a block in the development of multiple progenitors.[34,35]

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

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-inositide-3 kinase (PI3K) signaling pathways through *PTEN* or *FoxO1/3/4* deletion using the *Mx1-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 *FoxO*1/3/4 knockout but not in the *PTEN* knockout.[37] Ultimately, both animals acquire a myleoproliferative syndrome or leukemia, but the defects in HSC proliferation and function are temporally and clonally separable from these diseases.

Lastly, an *Mx1-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 *FoxO*1/3/4 knockout.[37,41] 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 allow 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.[42,43]

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 were demonstrated to influence the expression of their target genes in a manner that is transmissible through daughter cell divisions.[44,45] Mammalian genes related to both groups have been implicated in leukemia and other cancers.[46]

Bmi-1 represents one of the best-characterized Polycomb family members with respect to function within multiple stem cell types. *Bmi-1*-deficient mice exhibit a reduction in phenotypically-defined HSCs; these cells engraft poorly and exhaust prematurely in serial transplantation experiments. Both gain- and loss-of-function experiments have demonstrated that *Bmi-1* plays a role in HSC self-renewal, with an initial focus on the role of *Bmi-1* as a negative regulator of the CDK inhibitor, p16^{INK4A} [47-49]

Several other Polycomb family members, *M33, Mel-18* and *Rae28*, have also been shown to be important for maintaining HSC function.[50-52] Cell cycle defects are not apparent in most of these loss-of-function models, but some studies report observations consistent with the premature or ectopic activation of a senescence pathway,[53] although additional relevant processes are likely to be affected.

One of our groups (Jude et al.) and McMahon et al.,[54,55] have recently placed the TrG protein, MLL, in this category. Both studies demonstrated a reduction in HSC number and function using distinct *Cre* transgenes and gene disruption strategies and identified a role for *Mll* in maintaining quiescent versus cycling HSCs. Jude et al. demonstrated that upon *Mll* deletion, the LSK/CD48- subpopulation (enriched in quiescent cells) exhibited ectopic entry into G_1. Shortly thereafter, an increased number of cells in S-phase were observed in the total LSK population based on BrdU labeling studies.[54] Although these observations predict the type of transient accumulation in LSK cells as described in the previous section for β-catenin activation or *Myc* loss, no such increase was observed. This suggests that the increase in cycling HSCs was tightly coupled to the differentiation of these cells, thereby eliminating self-renewing cell divisions. A function for MLL in the negative regulation of proliferation specifically in HSCs was surprising, as MLL fusion oncoproteins are generally though to act by a gain-of-function mechanism.[56] Therefore the simplest prediction for the normal role of MLL during hematopoiesis might have been that it maintains the expression of pro-proliferative target genes, which are then hyper-activated or maintained inappropriately in the context of oncogenic MLL fusion proteins. However, the finding that *Mll* loss leads to enhanced entry into the cell cycle may indicate that a distinct HSC-specific set of *Mll* target genes coordinate the proper balance of proliferation versus quiescence.

The constitutive knockout of the histone acetyltransferase MOZ results in some features in common with the *Mll* knockouts described above, likely due to its participation in some common genetic pathways. MOZ (encoded by the *MYST3* gene) was identified at the site of the 8;16 translocation in AML and has been implicated in the transactivation function of *Runx1*.[57] *Myst3*, like *Mll*, positively regulates *Hoxa9*.[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 *Hoxa* 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 *Mx1-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

©2008 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

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-activation mutants, where an independent block in the development of lymphoid and megakaryocytic, or erythroid, myeloid and lymphoid lineages, respectively, is clearly demonstrated.[28-31,33] In addition to performing careful 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.[63] 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 G_0/G_1 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 process 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

1. Sherr CJ. Cancer cell cycles. Science 1996; 274:1672-7.
2. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004; 10:789-99.
3. Rosenbauer F, Tenen DG. Transcription factors in myeloid development: balancing differentiation with transformation. Nat Rev Immunol 2007; 7:105.
4. Walkley CR, McArthur GA, Purton LE. Cell division and hematopoietic stem cells: not always exhausting. Cell Cycle 2005; 4:893-6.
5. Mucenski ML, McLain K, Kier AB, Swerdlow SH, Schreiner CM, Miller TA, Pietryga DW, Scott WJ, Jr., Potter SS. A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. Cell 1991; 65:677-89.
6. Emambokus N, Vegiopoulos A, Harman B, Jenkinson E, Anderson G, Frampton J. Progression through key stages of haemopoiesis is dependent on distinct threshold levels of c-Myb. Embo J 2003; 22:4478-88.
7. Sakamoto H, Dai G, Tsujino K, Hashimoto K, Huang X, Fujimoto T, Mucenski M, Frampton J, Ogawa M. Proper levels of c-Myb are discretely defined at distinct steps of hematopoietic cell development. Blood 2006; 108:896-903.
8. Kasper LH, Boussouar F, Ney PA, Jackson CW, Rehg J, van Deursen JM, Brindle PK. A transcription-factor-binding surface of coactivator p300 is required for haematopoiesis. Nature 2002; 419:738-43.
9. Rebel VI, Kung AL, Tanner EA, Yang H, Bronson RT, Livingston DM. Distinct roles for CREB-binding protein and p300 in hematopoietic stem cell self-renewal. Proc Natl Acad Sci USA 2002; 99:14789-94.
10. Sandberg ML, Sutton SE, Pletcher MT, Wiltshire T, Tarantino LM, Hogenesch JB, Cooke MP. c-Myb and p300 regulate hematopoietic stem cell proliferation and differentiation. Dev Cell 2005; 8:153-66.
11. Morrison SJ, Lagasse E, Weissman IL. Demonstration that Thy(lo) subsets of mouse bone marrow that express high levels of lineage markers are not significant hematopoietic progenitors. Blood 1994; 83:3480-90.
12. Antonchuk J, Sauvageau G, Humphries RK. HOXB4 overexpression mediates very rapid stem cell regeneration and competitive hematopoietic repopulation. Experimental Hematology 2001; 29:1125.
13. Fukushima T, Miyazaki Y, Tsushima H, Tsutsumi C, Taguchi J, Yoshida S, Kuriyama K, Scadden D, Nimer S, Tomonaga M. The level of MEF but not ELF-1 correlates with FAB subtype of acute myeloid leukemia and is low in good prognosis cases. Leuk Res 2003; 27:387-92.
14. Mao S, Frank RC, Zhang J, Miyazaki Y, Nimer SD. Functional and physical interactions between AML1 proteins and an ETS protein, MEF: implications for the pathogenesis of t(8;21)-positive leukemias. Mol Cell Biol 1999; 19:3635-44.
15. Lacorazza HD, Yamada T, Liu Y, Miyata Y, Sivina M, Nunes J, Nimer SD. The transcription factor MEF/ELF4 regulates the quiescence of primitive hematopoietic cells. Cancer Cell 2006; 9:175-87.
16. Hock H, Hamblen MJ, Rooke HM, Schindler JW, Saleque S, Fujiwara Y, Orkin SH. Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. Nature 2004; 431:1002-7.
17. Zeng H, Yucel R, Kosan C, Klein-Hitpass L, Moroy T. Transcription factor Gfi1 regulates self-renewal and engraftment of hematopoietic stem cells. Embo J 2004; 23:4116-25.
18. Schmidt T, Zornig M, Beneke R, Moroy T. MoMuLV proviral integrations identified by Sup-F selection in tumors from infected myc/pim bitransgenic mice correlate with activation of the gfi-1 gene. Nucleic Acids Res 1996; 24:2528-34.
19. Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. Science 1995; 269:1427-9.
20. Passegue E. Unpublished Observation. 2007.
21. Passegue E, Wagner EF, Weissman IL. JunB deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. Cell 2004; 119:431-43.
22. Gonda TJ, Sheiness DK, Bishop JM. Transcripts from the Cellular Homologs of Retroviral Oncogenes: Distribution Among Chicken Tissues. Mol Cell Biol 1982; 2:617-24.
23. Grisendi S, Pandolfi PP. Two Decades of Cancer Genetics: From Specificity to Pleiotropic Networks. Cold Spring Harbor Symposia on Quantitative Biology 2005; 70:83-91.
24. Baena E, Ortiz M, Martinez-A C, Moreno de Alboran I. c-Myc is essential for hematopoietic stem cell differentiation and regulates Lin-Sca-1+c-Kit- cell generation through p21. Experimental Hematology 2007; 35:1333.
25. Wilson A, Murphy MJ, Oskarsson T, Kaloulis K, Bettess MD, Oser GM, Pasche AC, Knabenhans C, Macdonald HR, Trumpp A. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev 2004; 18:2747-63.
26. Malynn BA, de Alboran IM, O'Hagan RC, Bronson R, Davidson L, DePinho RA, Alt FW. N-myc can functionally replace c-myc in murine development, cellular growth, and differentiation. Genes Dev 2000; 14:1390-9.
27. Meyers S, Lenny N, Hiebert SW. The t(8;21) fusion protein interferes with AML-1B-dependent transcriptional activation. Mol Cell Biol 1995; 15:1974-82.
28. Gaudet J, Speck NA. Unpublished Observation. 2007.
29. Growney JD, Shigematsu H, Li Z, Lee BH, Adelsperger J, Rowan R, Curley DP, Kutok JL, Akashi K, Williams IR, Speck NA, Gilliland DG. Loss of Runx1 perturbs adult hematopoiesis and is associated with a myeloproliferative phenotype. Blood 2005; 106:494-504.

©2008 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

30. Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, Mitani K, Chiba S, Ogawa S, Kurokawa M, Hirai H. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. Nat Med 2004; 10:299-304.
31. Putz G, Rosner A, Nuesslein I, Schmitz N, Buchholz F. AML1 deletion in adult mice causes splenomegaly and lymphomas. Oncogene 2005; 25:929.
32. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell 2005; 121:1109-21.
33. Harada N, Tamai Y, Ishikawa T, Sauer B, Takaku K, Oshima M, Taketo MM. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. Embo J 1999; 18:5931-42.
34. Kirstetter P, Anderson K, Porse BT, Jacobsen SEW, Nerlov C. Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. Nat Immunol 2006; 7:1048.
35. Scheller M, Huelsken J, Rosenbauer F, Taketo MM, Birchmeier W, Tenen DG, Leutz A. Hematopoietic stem cell and multilineage defects generated by constitutive [beta]-catenin activation. Nat Immunol 2006; 7:1037.
36. Trowbridge JJ, Scott MP, Bhatia M. Hedgehog modulates cell cycle regulators in stem cells to control hematopoietic regeneration. Proceedings of the National Academy of Sciences 2006; 103:14134-9.
37. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, McDowell EP, Lazo-Kallanian S, Williams IR, Sears C, Armstrong SA, Passegue E, DePinho RA, Gilliland DG. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 2007; 128:325-39.
38. Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, Haug JS, Rupp D, Porter-Westpfahl KS, Wiedemann LM, Wu H, Li L. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature 2006; 441:518-22.
39. Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M, Chen C, Hosokawa K, Nakauchi H, Nakayama K, Nakayama KI, Harada M, Motoyama N, Suda T, Hirao A. Foxo3a Is Essential for Maintenance of the Hematopoietic Stem Cell Pool. Cell Stem Cell 2007; 1:101-12.
40. Yilmaz ÒH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, Morrison SJ. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature 2006; 441:475.
41. Yang L, Wang L, Geiger H, Cancelas JA, Mo J, Zheng Y. Rho GTPase Cdc42 coordinates hematopoietic stem cell quiescence and niche interaction in the bone marrow. Proc Natl Acad Sci USA 2007; 104:5091-6.
42. Passegue E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. J Exp Med 2005; 202:1599-611.
43. Lambert JF, Liu M, Colvin GA, Dooner M, McAuliffe CI, Becker PS, Forget BG, Weissman SM, Quesenberry PJ. Marrow Stem Cells Shift Gene Expression and Engraftment Phenotype with Cell Cycle Transit. J Exp Med %R 101084/jem20030031 2003; 197:1563-72.
44. Cavalli G, Paro R. The Drosophila Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. Cell 1998; 93:505-18.
45. Cavalli G, Paro R. Epigenetic Inheritance of Active Chromatin After Removal of the Main Transactivator. Science 1999; 286:955-8.
46. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 2006; 6:846-56.
47. Iwama A, Oguro H, Negishi M, Kato Y, Morita Y, Tsukui H, Ema H, Kamijo T, Katoh-Fukui Y, Koseki H, van Lohuizen M, Nakauchi H. Enhanced self-renewal of hematopoietic stem cells mediated by the polycomb gene product Bmi-1. Immunity 2004; 21:843-51.
48. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature 2003; 423:255-60.
49. Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ, Clarke MF. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 2003; 423:302-5.
50. Core N, Bel S, Gaunt SJ, Aurrand-Lions M, Pearce J, Fisher A, Djabali M. Altered cellular proliferation and mesoderm patterning in Polycomb-M33-deficient mice. Development 1997; 124:721-9.
51. Kajiume T, Ninomiya Y, Ishihara H, Kanno R, Kanno M. Polycomb group gene mel-18 modulates the self-renewal activity and cell cycle status of hematopoietic stem cells. Exp Hematol 2004; 32:571-8.
52. Ohta H, Sawada A, Kim JY, Tokimasa S, Nishiguchi S, Humphries RK, Hara J, Takihara Y. Polycomb group gene rae28 is required for sustaining activity of hematopoietic stem cells. J Exp Med 2002; 195:759-70.
53. Iwama A, Oguro H, Negishi M, Kato Y, Nakauchia H. Epigenetic regulation of hematopoietic stem cell self-renewal by polycomb group genes. Int J Hematol 2005; 81:294-300.
54. Jude CD, Climer L, Xu D, Artinger E, Fisher JK, Ernst P. Unique and Independent Roles for MLL in Adult Hematopoietic Stem Cells and Progenitors. Cell Stem Cell 2007; 1:324-37.
55. McMahon KA, Hiew SY-L, Hadjur S, Veiga-Fernandes H, Menzel U, Price AJ, Kioussis D, Williams O, Brady HJM. Mll Has a Critical Role in Fetal and Adult Hematopoietic Stem Cell Self-Renewal. Cell Stem Cell 2007; 1:338-45.
56. Daser A, Rabbitts TH. Extending the repertoire of the mixed-lineage leukemia gene MLL in leukemogenesis. Genes Dev 2004; 18:965-74.
57. Kitabayashi I, Aikawa Y, Nguyen LA, Yokoyama A, Ohki M. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. Embo J 2001; 20:7184-96.
58. Dou Y, Milne TA, Tackett AJ, Smith ER, Fukuda A, Wysocka J, Allis CD, Chait BT, Hess JL, Roeder RG. Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. Cell 2005; 121:873-85.
59. Thomas T, Corcoran LM, Gugasyan R, Dixon MP, Brodnicki T, Nutt SL, Metcalf D, Voss AK. Monocytic leukemia zinc finger protein is essential for the development of long-term reconstituting hematopoietic stem cells. Genes Dev 2006; 20:1175-86.
60. Katsumoto T, Aikawa Y, Iwama A, Ueda S, Ichikawa H, Ochiya T, Kitabayashi I. MOZ is essential for maintenance of hematopoietic stem cells. Genes Dev 2006; 20:1321-30.
61. Kang-Decker N, Tong C, Boussouar F, Baker DJ, Xu W, Leontovich AA, Taylor WR, Brindle PK, van Deursen JM. Loss of CBP causes T cell lymphomagenesis in synergy with p27Kip1 insufficiency. Cancer Cell 2004; 5:177-89.
62. Oguro H, Iwama A, Morita Y, Kamijo T, van Lohuizen M, Nakauchi H. Differential impact of Ink4a and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. J Exp Med 2006; 203:2247-53.
63. Wright DE, Wagers AJ, Gulati AP, Johnson FL, Weissman IL. Physiological migration of hematopoietic stem and progenitor cells. Science 2001; 294:1933-6.
64. Abkowitz JL, Robinson AE, Kale S, Long MW, Chen J. Mobilization of hematopoietic stem cells during homeostasis and after cytokine exposure. Blood 2003; 102:1249-53.
65. McKinney-Freeman S, Goodell MA. Circulating hematopoietic stem cells do not efficiently home to bone marrow during homeostasis. Exp Hematol 2004; 32:868-76.

©2008 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.