Fine-tuning PU.1

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The transcription factor PU.1 has an essential role in hematopoiesis. New evidence shows that reducing PU.1 expression to 20% of wild-type levels results in an aggressive form of acute myeloid leukemia.

PU.1, encoded by the gene *Sfpi1*, belongs to the Ets family of transcription factors¹. Its expression is tightly controlled in hematopoietic cells, such that the relative expression level of PU.1 determines whether cells differentiate into lymphocytes, macrophages or granulocytes². Mutations in *FLT3* and *RUNX1-CBFA2T1* translocations, which are commonly found in acute myeloid leukemia (AML), were recently found to downregulate expression of PU.1, causing a block in differentiation and implicating PU.1 in leukemogenesis^{3,4}. A new study by Frank Rosenbauer and colleagues⁵ now shows that reducing PU.1 levels below a critical threshold in mice results in an aggressive form of AML, identifying a dosage sensitivity for this lineage-specific transcription factor during leukemic progression.

Adjusting the dose

Dan Tenen and colleagues previously identified a key upstream regulatory region in *Sfpi1* that controls levels of PU.1 expression⁶. In this new study, Rosenbauer *et al.* replaced the upstream regulatory region with a neomycin resistance cassette and generated mice carrying a modified, knockdown *Sfpi1* allele. Mice homozygous with respect to the knockdown allele expressed PU.1 at 20% of wild-type levels and had more, poorly differentiated myeloid progenitors. This block in differentiation was suggested to coincide with a preleukemic state. After a short latency period (3–8 months), most mice developed a disease resembling AML. Inoculation of leukemic splenocytes from PU.1-knockdown mice into nonirradiated NOD/SCID and wild-type mice also caused leukemia, indicating that the leukemic phenotype was transferable.

This aggressive, malignant transformation of PU.1-knockdown cells is in stark contrast to previous work examining the effects of complete loss or 50% reduction of PU.1 expression. In particular, complete loss of

PU.1 causes a severe dysfunction of hematopoiesis, with mice dying from infections or other nonmalignant etiologies^{7,8}, whereas 50% reduction results in normal hematopoiesis⁷. Rosenbauer *et al.* hypothesize that the low level of PU.1 promotes a preleukemic state by disrupting normal responsiveness to growth factors. In fact, preleukemic PU.1-knockdown cells lacked expression of M-CSF and GM-CSF receptors but maintained normal expression of the G-CSF receptor. This is in contrast to normal hematopoietic cells, which express all three

receptors, and cells devoid of PU.1, which have markedly reduced expression of all three^{2,9}. Not surprisingly, treatment of preleukemic PU.1-knockdown cells with M-CSF and GM-CSF did not promote differentiation, whereas treatment with G-CSF did, indicating that PU.1-knockdown cells retain some normal hematopoietic responses.

Rosenbauer *et al.* further postulate that additional genetic abnormalities are needed to transform preleukemic PU.1-knockdown cells into a leukemic phenotype (Fig. 1). Most leukemic PU.1 knockdown cells that they

Figure 1 PU.1 dosage effect in leukemogenesis. Normal PU.1 expression (wild-type PU.1, 100%) promotes expression of GM-CSF (GM), G-CSF (G) and M-CSF (M) receptors on the surface of hematopoietic cells, which regulate differentiation of primitive hematopoietic cells. When PU.1 is expressed at 20% of wild-type levels (PU.1 knockdown, 20%), cells lose expression of GM-CSF and M-CSF receptors but retain expression of the G-CSF receptor. These cells have abnormal proliferation and differentiation, leading to an accumulation of cells in a preleukemic state. Additional mutations cause progression to a more aggressive, leukemic phenotype. When PU.1 is completely absent (PU.1 null, 0%), GM-CSF, G-CSF and M-CSF receptors are not expressed and hematopoietic differentiation is blocked. This leads to a profound disruption of normal hematopoiesis, resulting in premature death from infections or other nonmalignant causes.

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analyzed (five of seven) showed additional chromosomal abnormalities, with amplification of the chromosome 15 region harboring *Myc* observed in four of seven cases.

Normal hematopoiesis requires the precise regulation of multiple pathways whose activities fluctuate during proliferation and differentiation. Individual genes in these pathways are like 'on-off' switches that dictate the level of activity in a given pathway, and investigators hypothesize that leukemogenesis results from an absolute disruption in one or more of these switches. Such errors are usually associated with activating mutations that turn the switch on at the wrong time (*RAS*, *FLT3*) or with the creation of new chimeric proteins (Bcr-Abl, PML-RAR α , RUNX1-CBFA2T1) that perturb the regulatory system. In the 1990s, however, numerous studies showed that loss of heterozygosity of some tumor suppressor genes can promote malignant transformation, suggesting that a haploinsufficient dosage effect may have a role in tumorigenesis^{10,11}.

Rosenbauer *et al.* expanded on this concept by showing that a dosage effect for PU.1 exists between haploinsufficient and null expression states. These results suggest that investigators must look beyond the concept of genes merely acting as on-off switches and begin considering how small fluctuations in activity impact the biology of the cell. Looking to the future, research will need to more accurately define the interactions between genes and molecular pathways to develop a clearer picture of what constitutes the 'normal' and

'abnormal' regulatory processes of cells. These investigations will necessitate the development of new quantitative approaches and improvements in existing technologies such as DNA microarrays and proteomics.

The elaborate networks controlling cellular functions have multiple checks and balances. For example, *RAS* mutations, which are constitutively active, upregulate p53 activity, resulting in apoptosis if the p53 tumor suppressor pathway remains functional¹². Therefore, it is not surprising that multiple genetic 'hits' are necessary to promote leukemogenesis. Recently, investigators found that mutations in *FLT3* can cooperate with PML-RAR α rearrangements to promote rapid development of a leukemia-like disease in a transgenic mice, whereas either genetic abnormality alone induces primarily a myeloproliferative-like syndrome¹³. These results, combined with those of Rosenbauer *et al.*, argue that initial genetic events promote an undifferentiated or preleukemic state that makes cells susceptible to additional genetic damage. The phenotype of this preleukemic state is similar to that found in myeloproliferative diseases, in which there is an expansion of the primitive hematopoietic cell compartment. Additional genetic abnormalities, like *FLT3* mutations or overexpression of c-Myc, push these cells into an aggressive leukemic phenotype. These findings are consistent with the multiple-hit theory of carcinogenesis, first proposed for solid tumors such as colon cancer¹⁴.

The primary clinical implications from these findings are that cells in a preleukemic

state retain some normal regulatory pathways. Therefore, these cells should be much more susceptible, in theory, to targeted therapies that induce preleukemic cells into a normal pathway of differentiation, and perhaps apoptosis. Once cells have evolved to a leukemic state, many of these regulatory processes have been by-passed, making targeted approaches less likely to succeed. This concept has been clearly validated in chronic myelogenous leukemia, in which the small molecular inhibitor Imatinib is effective in treating chronic myelogenous leukemia in chronic phase but has limited efficacy once the disease has progressed to blast phase, a more aggressive and overtly leukemic state¹⁵.

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Plzf pushes stem cells

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The molecular mechanisms that regulate the balance between differentiation and self-renewal in spermatogonial stem cells are elusive. Two studies now show that the transcriptional repressor Plzf is an essential regulator of spermatogonial stem cell maintenance.

Among cell lineages, germ cells are unique in that they can generate a new organism. In males, germline stem cells provide a source of undifferentiated cells that allow spermatogenesis to proceed throughout the period of sexual maturity. Cells committed to differentiate enter the meiotic

pathway, which comprises a unique program of gene expression and chromatin remodeling¹. To maintain the stem cell pool, however, some germ cells must remain undifferentiated and proliferate through cyclic mitotic divisions. How does each spermatogonial stem cell decide whether to proliferate or differentiate? The molecular mechanisms controlling this delicate balance are largely unknown. New studies by F. William Buaas and colleagues and José Costoya and colleagues published

in this issue^{2,3} provide clues to the mechanisms required for self-renewal of spermatogonial stem cells by showing that the transcriptional repressor Plzf is required for stem cell maintenance.

An epigenetic connection

Plzf (promyelocytic leukemia zinc-finger) belongs to the POK (POZ and Krüppel) family of transcriptional repressors. In addition to nine Krüppel-type sequence-specific zinc fingers, Plzf contains a conserved POZ

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