Selected reading

Bao, S., Wu, Q., McLendon, R.E., Hao, Y., Shi, Q., Hjelmeland, A.B., Dewhirst, M.W., Bigner, D.D., and Rich, J.N. (2006). Nature. Published online October 18, 2006. 10.1038/nature05236.

Galli, R., Binda, E., Orfanelli, U., Cipelletti, B., Gritti, A., De Vitis, S., Fiocco, R., Foroni, Ch., Dimeco, F., and Vescovi, A. (2004). Cancer Res. *64*, 7011–7021.

Holland, E.C., Celestino, J., Dai, C., Schaefer, L., Sawaya, R.E., and Fuller, G.N. (2000). Nat. Genet. *25*, 55–57.

Hornsey, S. (1973). Strahlenschutz Forsch. Prax. *13*, 78–88.

Marshman, E., Booth, C., and Potten, C.S. (2002). Bioessays *24*, 91–98.

McCulloch, E.A., and Till, J.E. (1960). Radiat. Res. *13*, 115–125.

McCulloch, E.A., and Till, J.E. (2005). Nat. Med. *10*, 1026–1028.

Rich, J.N., Sathornsumetee, S., Keir, S.T., Kieran, M.W., Laforme, A., Kaipainen, A., McLendon, R.E., Graner, M.W., Rasheed, B.K., Wang, L., et al. (2005a). Clin. Cancer Res. *11*, 8145–8157.

Rich, J.N., Hans, C.H., Jones, B., Iverson, E.S., McLendon, R.E., Rasheed, B.K., Dobra, A., Dressman, H.K., Bigner, D.D., Nevins, J.R., and West, M. (2005b). Cancer Res. *65*, 4051–4058.

Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D., and Dirks, P.B. (2004). Nature *432*, 396–401.

DOI 10.1016/j.ccr.2006.11.008

PU.1 and Junb: Suppressing the formation of acute myeloid leukemia stem cells

Improved understanding of the molecular pathways that suppress the genesis and maintenance of cancer stem cells will facilitate development of rationally targeted therapies. PU.1 is a transcription factor that is required for normal myelomonocytic differentiation in hematopoiesis, and reduced PU.1 activity has been associated with myeloid leukemogenesis in man and in mouse models. A recent study by Steidl et al. demonstrates that Junb and Jun, two AP-1 transcription factors, are critical downstream effectors of the tumor suppressor activity of PU.1, and that reduced expression of *Junb*, in particular, may be a common feature of acute myeloid leukemogenesis.

Tissue-specific stem cells are considered fertile soil for some of the mutations that contribute to the development of human cancers. However, the molecular mechanisms by which these mutations give rise to cancer stem cells, or otherwise lead to neoplastic disease, are less well defined. This issue is of major importance, since the implicated pathways may be targets for molecular therapies, particularly if they are selectively involved in cancer versus normal stem cell maintenance.

Of the many molecular pathologies associated with acute myeloid leukemia (AML), one recurrently implicated gene is *PU.1* (*SPI1*; *Sfpi1*, for Spleen focus-forming virus proviral integration), which codes for a transcription factor that is essential for normal myelomonocytic differentiation and consequently also functions as a tumor suppressor (reviewed in Koschmieder et al., 2005). Repressed *PU.1* transcription has been reported in AMLs harboring *PML-RAR*α (Mueller et al., 2006) or *FLT3-ITD* mutations, and the AML-associated oncoprotein AML1-ETO functionally inactivates PU.1 through displacement of its coactivator, JUN (Koschmieder et al., 2005). Heterozygous mutations of *PU.1* have been observed in one series of patients with AML and are postulated to co-operate with reduced PU.1 activity induced by other mechanisms to promote leukemogenesis (Koschmieder et al., 2005). Consistent with these observations, reduced or abrogated *PU.1* expression in mouse models results in AML (Rosenbauer et al., 2004; Metcalf et al., 2006). Thus, PU.1 activity appears to be a target of several oncogenic signaling pathways in AML (Figure 1). However, the downstream genes that are critical mediators of its leukemia-suppressive role have hitherto been undefined.

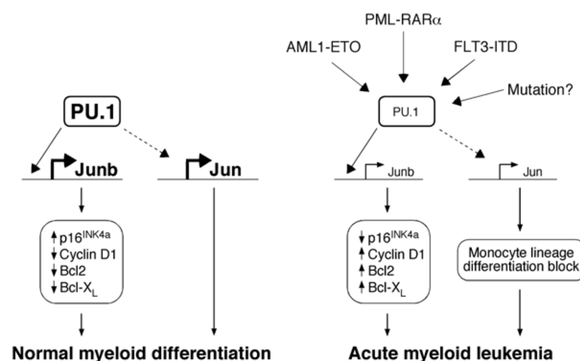

Figure 1. PU.1 in normal and leukemic hematopoiesis

In normal hematopoiesis, PU.1 promotes myelomonocytic differentiation through positively regulating expression of the AP-1 transcription factors Junb and Jun. In acute myeloid leukemia, a variety of mechanisms contribute to a reduction in PU.1 activity, leading to reduced *Junb* and *Jun* expression, with consequent dysregulation of differentiation, programmed cell death, and cellular proliferation.

In an elegant series of experiments, Steidl et al. (2006) have recently solved a significant piece of this puzzle by identifying Jun and Junb, members of the activator protein-1 (AP-1) family of transcriptional regulators, as critical effectors of the PU.1 tumor suppressor pathway. Their studies employed a PU.1 knockdown (PU.1 KD) mouse model that develops highly penetrant AML as a consequence of reduced *PU.1* expression caused by deletion of a critical upstream regulatory element in the *PU.1* gene. Global transcriptional analysis of an immature subfraction of bone marrow cells obtained from preleukemic PU.1 KD mice identified *Jun* and *Junb*, in addition to a number of previously known PU.1 targets, to be downregulated compared with cells obtained from wild-type mice. The compared cell populations,

so-called KSL cells (Kit$^+$, Sca1$^+$, Lin$^-$), normally contain about 15% long-term hematopoietic stem cells (HSCs) as well as other transiently reconstituting multipotent progenitors. When these cells were isolated from leukemic PU.1 KD donor mice and transplanted into immunocompromised recipient mice, the recipients developed AML. Thus, at least a proportion of cells with this immunophenotype in leukemic PU.1 KD mice are leukemia stem cells (LSCs) that display significantly downregulated *Jun* and *Junb* expression by comparison with normal KSL cells.

Steidl et al. further demonstrated that *Junb* is a direct target gene for PU.1, which bound to and regulated expression of *Junb* through a conserved upstream DNA element. Interestingly, *Junb* itself has been shown to be a tumor suppressor in myelopoiesis. Mice lacking *Junb* expression in HSCs develop a myeloproliferative disease similar to human chronic myeloid leukemia (CML) (Passegue et al., 2004), and methylation-induced silencing of *Junb* occurs in cells from chronic phase and blastic transformation of CML (Yang et al., 2003). Taken together, these observations suggest that direct regulation of *Junb* by PU.1 may constitute a critical transcriptional circuit for suppression of myeloid leukemogenesis (Figure 1).

To directly test this possibility, *Junb* was forcibly expressed in PU.1 KD leukemia cells in an effort to bypass the oncogenic effects of reduced *PU.1* expression. This antagonized the oncogenic properties of PU.1 KD AML cells as evidenced by reduced clonogenic potential, serial replating activity, and proliferation in liquid culture, whereas clonogenic potentials of normal bone marrow progenitor cells were unaffected. The effects were specific to *Junb*, because similar forced expression of *Jun* did not block AML cell proliferation. Furthermore, forced expression of *Junb* in PU.1 KD leukemia cells inhibited their ability to induce AML in secondary recipients, indicating that restoration of *Junb* expression was sufficient to abrogate LSC activity associated with PU.1 knockdown.

To test the relevance for human disease, the authors interrogated an AML global gene expression data set and discovered that *PU.1* and *JUNB* expression were very significantly correlated with each other, particularly in the AML-M4 and M5 subtypes. Prospective isolation of primitive hematopoietic progenitor cells (CD34$^+$, CD38$^-$, CD90^{low}, Lin$^-$) from AML patients showed that *PU.1* and *JUNB* transcript levels were also highly correlated in a phenotypic population reportedly enriched for human LSC activity. Furthermore, when compared with normal progenitor cells with a similar phenotype, *JUNB* transcript levels were lower in the LSC-enriched populations. However, the higher prevalence of reduced *JUNB* expression (17/20 cases) compared with reduced *PU.1* expression (7/20 cases) in LSC-enriched populations compared with normal control populations also raised the intriguing possibility that a variety of mechanisms may account for impaired maintenance of *JUNB* expression other than reduced PU.1 activity. These data support a critical role for *PU.1* and *JUNB* as myeloid lineage tumor suppressors and suggest that dysregulation of this pathway may frequently occur in human leukemic stem cells.

The authors' studies raise two interesting questions. First, if loss of *Junb* expression is a critical downstream effect of PU.1 knockdown, why do PU.1 KD mice develop AML rather than the myeloproliferative disease observed when *Junb* is inactivated in long-term HSCs (Passegue et al., 2004)? One likely explanation is that reduced *PU.1* expression has pleiotropic downstream effects. Consistent with this, Steidl et al. observed that in preleukemic PU.1 KD mice there is a block in terminal monocyte/macrophage lineage differentiation, which is reversed by expression of *Jun*. It is possible, therefore, that impaired differentiation induced by *Jun* deficiency and increased proliferation induced by *Junb* deficiency collaborate to induce the observed disease morphology. Secondly, how might reduced *Junb* expression promote myeloid leukemogenesis? Although not formally investigated in this study, Junb has previously been shown to repress cyclin D1, Bcl2, and Bcl-X_L expression and to activate expression of the cyclin-dependent kinase inhibitor p16^{INK4a} (Passegue et al., 2001), which together may alter the apoptotic rheostat in favor of cell death as well as promoting cell cycle exit to facilitate normal myeloid differentiation (Figure 1). Loss of *Junb* would favor the reverse processes.

Through their analysis of an interesting murine genetic model of AML, Steidl et al. have defined an important transcriptional pathway that may well be dysregulated in human AML stem cells, which are the cells that must be eliminated by therapy in order to cure disease. These and other recent studies (for example, Somervaille and Cleary, 2006; Krivtsov et al., 2006) validate the use of murine models to further our understanding of LSCs and pertinent cellular pathways relevant for human AML. Their studies also add further promise that the molecular pathways underlying LSC maintenance may be selectively targeted while sparing normal HSCs.

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Selected reading

Koschmieder, S., Rosenbauer, F., Steidl, U., Owens, B.M., and Tenen, D.G. (2005). Int. J. Hematol. *81*, 368–377.

Krivtsov, A.V., Twomey, D., Feng, Z., Stubbs, M.C., Wang, Y., Faber, J., Levine, J.E., Wang, J., Hahn, W.C., Gilliland, D.G., et al. (2006). Nature *442*, 818–822.

Metcalf, D., Dakic, A., Mifsud, S., Di Rago, L., Wu, L., and Nutt, S. (2006). Proc. Natl. Acad. Sci. USA *103*, 1486–1491.

Mueller, B.U., Pabst, T., Fos, J., Petkovic, V., Fey, M.F., Asou, N., Buergi, U., and Tenen, D.G. (2006). Blood *107*, 3330–3338.

Passegue, E., Jochum, W., Schorpp-Kistner, M., Mohle-Steinlein, U., and Wagner, E.F. (2001). Cell *104*, 21–32.

Passegue, E., Wagner, E.F., and Weissman, I.L. (2004). Cell *119*, 431–443.

Rosenbauer, F., Wagner, K., Kutok, J.L., Iwasaki, H., Le Beau, M.M., Okuno, Y., Akashi, K., Fiering, S., and Tenen, D.G. (2004). Nat. Genet. *36*, 624–630.

Somervaille, T.C., and Cleary, M.L. (2006). Cancer Cell *10*, 257–268.

Steidl, U., Rosenbauer, F., Verhaak, R.G., Gu, X., Ebralidze, A., Otu, H.H., Klippel, S., Steidl, C., Bruns, I., Costa, D.B., et al. (2006). Nat. Genet. *38*, 1269–1277.

Yang, M.Y., Liu, T.C., Chang, J.G., Lin, P.M., and Lin, S.F. (2003). Blood *101*, 3205–3211.

DOI 10.1016/j.ccr.2006.11.009