Michelle I. Lin and
William C. Sessa*

Yale University School of Medicine
Vascular Cell Signaling and Therapeutics Program
Boyer Center for Molecular Medicine
295 Congress Avenue
New Haven, Connecticut 06536
*E-mail: william.sessa@yale.edu

Selected reading

Abramovitch, R., Dafni, H., Smouha, E., Benjamin, L.E., and Neeman, M. (1999). Cancer Res. *59*, 5012–5016.

Baluk, P., Lee, C.G., Link, H., Ator, E., Haskell, A., Elias, J.A., and McDonald, D.M. (2004). Am. J. Pathol. *165*, 1071–1085.

Benjamin, L.E., Golijanin, D., Itin, A., Pode, D., and Keshet, E. (1999). J. Clin. Invest. *103*, 159–165.

Inai, T., Mancuso, M., Hashizume, H., Baffert, F., Haskell, A., Baluk, P., Hu-Lowe, D.D., Shalinsky, D.R., Thurston, G., Yancopoulos, G.D., and McDonald, D.M. (2004). Am. J. Pathol. *165*, 35–52.

Jain, R.K. (2001). Nat. Med. *7*, 987–989.

Jain, R.K. (2003). Nat. Med. *9*, 685–693.

Kerbel, R., and Folkman, J. (2002). Nat. Rev. Cancer *2*, 727–739.

Metheny-Barlow, L.J., and Li, L.Y. (2003). Cell Res. *13*, 309–317.

Papapetropoulos, A., Garcia-Cardena, G., Dengler, T.J., Maisonpierre, P.C., Yancopoulos, G.D., and Sessa, W.C. (1999). Lab. Invest. *79*, 213–223.

Thurston, G., Suri, C., Smith, K., McClain, J., Sato, T.N., Yancopoulos, G.D., and McDonald, D.M. (1999). Science *286*, 2511–2514.

Tong, R.T., Boucher, Y., Kozin, S.V., Winkler, F., Hicklin, D.J., and Jain, R.K. (2004). Cancer Res. *64*, 3731–3736.

Winkler, F., Kozin, S.V., Tong, R.T., Chae, S.S., Booth, M.F., Garkavtsev, I., Xu, L., Hicklin, D.J., Fukumura, D., di Tomaso, E., et al. (2004). Cancer Cell *6*, this issue.

Chronic versus acute myelogenous leukemia: A question of self-renewal

Leukemia stem cells are defined as transformed hematopoietic stem cells or committed progenitor cells that have amplified or acquired the stem cell capacity for self-renewal, albeit in a poorly regulated fashion. In this issue of *Cancer Cell*, Huntly and colleagues report a striking difference in the ability of two leukemia-associated fusion proteins, MOZ-TIF2 and BCR-ABL, to transform myeloid progenitor populations. This rigorous study supports the idea of a hierarchy among leukemia-associated protooncogenes for their ability to endow committed myeloid progenitors with the self-renewal capacity driving leukemic stem cell propagation, and sheds new light on the pathogenesis of chronic and acute myelogenous leukemias.

Human cancer stem cells, identified in acute myelogenous leukemia (Bonnet and Dick, 1997), myeloid blast crisis of chronic myelogenous leukemia (Jamieson et al, 2004), breast cancer (Al-Hajj et al., 2003), and brain tumors (Singh et al., 2003), share functional properties with normal stem cells, such as high proliferative potential, some differentiation capacity, and the ability to be serially transplanted (reviewed in Passegué et al., 2003). Signaling pathways involved in the regulation of normal stem cell self-renewal are frequently mutated or epigenetically activated in cancer, indicating that self-renewal, i.e. a cell division that produces progeny identical to the parental cell, is a vital property of cancer stem cells (reviewed in Reya et al., 2001). Targeted disruption of cancer stem cell self-renewal would represent a novel therapeutic strategy that could significantly reduce the capacity of a tumor to propagate itself, and could be employed in the eradication of a broad spectrum of cancers, including leukemias.

Chronic myelogenous leukemia (CML) and most types of acute myelogenous leukemia (AML) are induced by leukemia-associated fusion proteins that generally function as aberrantly activated signaling mechanisms or positive or negative transcriptional regulators, and directly interfere with the hematopoietic differentiation program. While their mechanism of action is relatively well understood, little is known about their developmental requirement for transformation and the role of self-renewal in this process. In this issue of *Cancer Cell*, Huntly et al. (2004) have studied in the mouse the target cell requirement of two human leukemia-associated fusion proteins, MOZ-TIF2 and BCR-ABL. MOZ-TIF2, an AML-associated fusion gene resulting from the inv (8)(p11q13)-induced juxtaposition of the MOZ chromatin remodeling gene and the TIF2 nuclear receptor transcriptional coactivator, is thought to modulate the transcriptional activity of target genes through aberrant histone acetylation. In contrast, BCR-ABL, the hallmark of CML, gives rise to a constitutively active protein tyrosine kinase, which endows primitive stem and progenitor cells with a proliferative and survival advantage (reviewed in Daley, 2004). Using retroviral gene transfer combined with in vitro serial replating assays and leukemic transplantation into lethally irradiated recipient mice, they compared the capacity of MOZ-TIF2 and BCR-ABL to enhance the self-renewal potential of normal murine bone marrow mononuclear cells, highly purified hematopoietic stem cells (HSC), and more committed progenitors including common myeloid progenitors (CMP) and granulocyte-monocyte progenitors (GMP). They also included critical controls, such as MOZ-TIF2 point mutants that lacked transforming activity, to exclude a contribution by retroviral insertional mutagenesis to the observed leukemogenic effects. The results presented in this paper demonstrate that MOZ-TIF2, but not BCR-ABL, endows myeloid progenitors with self-renewal

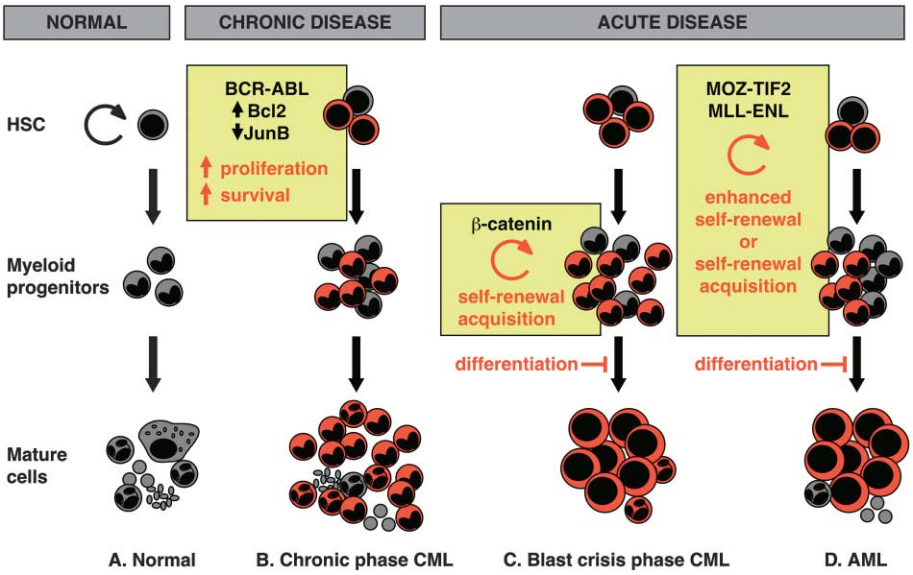

Figure 1. Oncogene hierarchy and role of self-renewal in pathogenesis of leukemias

A: Normal myelopoiesis is distinguished by the orderly differentiation of hematopoietic stem cells (HSC), which are the only cells with self-renewal capacity, into committed myeloid progenitors and their respective terminally differentiated progeny.

B: Chronic diseases, such as chronic phase CML, are associated with preleukemic events that result in increased survival and proliferation within the stem and myeloid progenitor populations but continued production of terminally differentiated progeny. Acquisition of BCR-ABL, overexpression of Bcl2, and inactivation of *Jun*B expression are examples of such events that initially take place in HSC.

C and D: Acute diseases such as blast crisis CML and AML are marked by acquisition of self-renewal capacity by progenitors that normally lack it or by enhanced self-renewal in HSC. β-catenin activation during the progression of CML to blast crisis is an example of such a leukemogenic event occurring in myeloid progenitors. MOZ-TIF2 and MLL-ENL are AML-associated translocation products that may enhance HSC self-renewal or endow myeloid progenitors with self-renewal potential. Together with a subsequent block in differentiation, these leukemogenic events result in the accumulation of immature blast progeny and development of AML at either the HSC or myeloid progenitor stage.

capacity, which leads to their immortalization in vitro and to their ability to give rise to AML following transplantation in vivo. Interestingly, MLL-ENL, another AML-associated fusion protein, also contributes to the transformation of committed progenitors that normally lack self-renewal capacity (Cozzio et al., 2003), further underscoring the importance of acquisition of self-renewal potential as a critical leukemogenic event that supersedes other factors such as the stage of commitment of the target cell population. By extending such studies to other AML-associated fusion genes, it should be possible to determine whether capacity to confer self-renewal potential is a defining criterion for oncogenes involved in acute-phase diseases. However, whether MOZ-TIF2 and MLL-ENL result in the activation of the same self-renewal target genes as those found for other leukemia-associated translocation products (Alcalay et al., 2003), or whether they induce self-renewal by independent means, remains to be determined.

BCR-ABL was the first leukemia-associated translocation product to be discovered and also the first target of molecular therapy involving the tyrosine kinase inhibitor imatinib. However, imatinib has not prevented CML progression in the majority of patients with advanced-phase disease. This is expected of single agent therapeutic targets wherein preexisting mutants or epigenetically altered gene products in cancer cells result in their emergence in a selection process, as first exemplified in microbial genetics (Luria and Delbruck, 1943; Lederberg, 1971). Resistance to imatinib in highly leukemic cells may also suggest that there are other potent transforming events that are not affected, and that the leukemia stem cell population harboring these events may be impervious to current forms of targeted therapy (reviewed in Daley, 2004). Huntly and colleagues demonstrate that leukemia-associated fusion proteins differ in their capacity to provide leukemogenic self-renewal potential, which supports the idea of a hierarchic order among the protooncogenic events that create leukemic stem cells from cells of different developmental origins. While MOZ-TIF2 is transforming at both the HSC and committed myeloid progenitor levels, BCR-ABL is incapable of transforming cells that lack inherent self-renewal capacity; this is in keeping with the previous characterization of chronic phase CML as a hematopoietic stem cell disease, and demonstrates that leukemogenic effects of individual oncogenes are cell-type- and context-specific (Jamieson et al., 2004; Passegué et al., 2004). The data presented by Huntly and coworkers suggest that BCR-ABL expression most likely represents a preleukemic event that provides a proliferative and survival advantage to CML stem and progenitor cells, but lacks the ability to endow self-renewal potential, and thus is not fully transforming when expressed in committed myeloid progenitors.

The pivotal work described in this paper also provides important insights into the multistep pathogenesis of leukemias (Figure 1) and helps to distinguish the molecular mechanisms controlling preleukemic events that impart a proliferative advantage and leukemic transforming events that confer a self-renewal advantage. In chronic phase CML, the preleukemic events most likely occur at the level of the HSC, since they are the only cells within the entire hematopoietic system that live long enough, due to self-renewal, to accumulate such rare events. Examples of preleukemic events include defects in proliferation and survival as a result of BCR-ABL expression, Bcl-2 overexpression or, as recently shown, loss of *Jun*B expression (Passegué et al., 2004). The emergence of an acute disease, such as blast crisis phase CML or AML, may occur when a preleukemic HSC subset or a population derived from preleukemic HSC gains self-renewal potential and emerges as a novel leukemic stem cell entity. Hence, a preleukemic phase, in which HSC

have acquired a number of mutations and/or epigenetic events providing them with a survival advantage, resistance to programmed cell death, and extended replicative lifespan, most likely facilitates the production of increased numbers of preleukemic clones that may gain aberrant self-renewal capacity. Amongst these preleukemic clones, the stage is now set for the inexorable progression to acute leukemia at either the HSC or myeloid progenitor stage. In human blast crisis CML, this leukemic evolution has been correlated with aberrant activation of the β-catenin pathway that endows preleukemic GMP with self-renewal potential and provides one of the final hits required for full leukemic transformation (Jamieson et al., 2004). Unlike BCR-ABL, genes such as MOZ-TIF2 or MLL-ENL can directly trigger self-renewal pathways in cells that normally lack them or enhance self-renewal in cells that have inherent self-renewal capacity. Together with their capacity to induce or enhance self-renewal, the ability of MOZ-TIF2 or MLL-ENL to block differentiation results in a much more potent oncogenic effect leading directly to the development of AML.

The elegant work performed by Huntly and colleagues emphasizes the critical role of self-renewal pathway activation in leukemia stem cell propagation and the importance of understanding these pathways and their effects at specific stages of hematopoietic development. Future therapies aimed at inhibiting the aberrant self-renewal capacity of leukemic stem cell populations may provide a potent means of preventing leukemic propagation, may be used in concert with other targeted therapies such as imatinib to ensure complete eradication of leukemia, and may be more broadly applicable in the treatment of other malignancies that have activated self-renewal pathways. Finally, a thorough understanding of molecular pathways involved in normal versus cancer stem cell self-renewal may provide important insights into the self-renewal process driving normal tissue regeneration, and consequently may have applications in tissue engineering.

Catriona H.M. Jamieson,[1,2,*] Irving L. Weissman,[1] and Emmanuelle Passegué[1]

[1]Institute of Cancer and Stem Cell Biology and Medicine, Departments of Pathology and Developmental Biology
[2]Division of Hematology, Department of Medicine
Stanford University School of Medicine
Stanford, California 94305
*E-mail: catriona@stanford.edu

Selected reading

Alcalay, M., Meani, N., Gelmetti, V., Fantozzi, A., Fagioli, M., Orleth, A., Riganelli, D., Sebastiani, C., Cappelli, E., Casciari, C., et al. (2003). J. Clin. Invest. *112*, 1751–1761.

Al-Hajj, M., Wicha, M., Benito-Hernandez, A., Morrison, S., and Clarke, M. (2003). Proc. Natl. Acad. Sci. USA *100*, 3983–3988.

Bonnet, D., and Dick, J.E. (1997). Nat. Med. *3*, 730–737.

Cozzio, A., Passegué, E., Ayton, P.M., Karsunky, H., Cleary, M.L., and Weissman, I.L. (2003). Genes Dev. *17*, 3029–3035.

Daley, G. (2004). Cell *119*, 314–316.

Huntly, B.J.P., Shigematsu, H., Deguchi, K., Lee, B.H., Mizuno, S., Duclos, N., Rowan, R., Amaral, S., Curley, D., Williams, I.R., et al. (2004). Cancer Cell *6*, this issue.

Jamieson, C.H., Ailles, L.E., Dylla, S.J., Muijtjens, M., Jones, C., Zehnder, J.L., Gotlib, J., Li, K., Manz, M.G., Keating, A., et al. (2004). N. Engl. J. Med. *351*, 657–667.

Lederberg, J. (1971). Science *173*, 104–105.

Luria, S., and Delbruck, M. (1943). Genetics *28*, 491–511.

Passegué, E., Jamieson, C.H.M., Ailles, L.E., and Weissman, I.L. (2003). Proc. Natl. Acad. Sci. USA *100*, 11842–11849.

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

Reya, T., Morrison, S., Clarke, M., and Weissman, I.L. (2001). Nature *414*, 105–111.

Singh, S.K., Clarke, I.D., Terasaki, M., Bonn, V.E., Hawkins, C., Squire, J., and Dirks, P.B. (2003). Cancer Res. *63*, 5821–5828.