IN THE SPOTLIGHT

Tracing the Roots of Cancer Evolution

Yanwen Jiang[1,2] and Olivier Elemento[1,3,4]

Summary: By comparing the genomes of progenitor cells and mature cells of lymphoid and myeloid lineages in patients with chronic lymphocytic leukemia (CLL), Damm and colleagues confirmed that CLL originates from preleukemic CD34+ progenitor cells and identified early CLL mutations that are associated with these progenitor cells. Moreover, they discovered that deregulation of B-cell receptor signaling may be one of the hallmarks of CLL, particularly in tumors with *EGR2* mutations. *Cancer Discov; 4(9); 995–7. ©2014 AACR.*

See related article by Damm et al., p. 1088 (7).

What is the cell of origin of tumors? This fundamental question has challenged cancer research for decades. Almost 30 years ago, in a review published in *Science*, Peter C. Nowell (1) proposed that tumors originate from a single cell and that "an induced change provides it with a selective growth advantage." Subsequently, "acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression." This groundbreaking theory, now known as the cancer clonal evolution model, was partly based on the observation he made with David Hungerford that in patients with chronic myelogenous leukemia (CML), essentially all tumor cells carried a chromosomal translocation (encoding *BCR–ABL*) known as the Philadelphia chromosome (1). Cancer as a clonal disease is now a widely accepted concept. However, the cell of origin of many cancers is still largely unknown, and the exact initial incident that triggers the cascade of events leading to malignant transformation is yet to be identified.

Hematologic malignancies provide great models to study the cell-of-origin question, largely owing to our relatively comprehensive knowledge of the hierarchical differentiation path from hematopoietic stem cell (HSC) to various lineages of blood cells where these malignancies arise. In addition, new technologies, such as multicolor flow cytometry and xenotransplantation in immune-deficient mouse strains, have helped hematologists pinpoint the rare cell populations that are responsible for the propagation of disease. For example, it is now evident that leukemia stem cells (LSC) exist in acute myelogenous leukemia (AML), and that these cells are capable of initiating and sustaining leukemic clones *in vivo* (2, 3). However, several key questions remain. First, the exact cell of origin of these LSCs is still up for debate. Two hypotheses have emerged. One proposes that LSCs originate from normal HSCs, given the phenotypic (cell-surface markers) and functional (self-renewal) similarities between these two cell populations. The other suggests that the initial transformation event occurs at lineage-restricted committed progenitor cells because of the phenotypic heterogeneity among patients with AML. Evidence from animal experiments has supported both models. For example, Huntly and colleagues (4) demonstrated that expression of the oncogenic AML fusion protein MOZ–TIF2 in common myeloid progenitors and granulocyte–monocyte progenitors (GMP) can recapitulate AML in mice. In contrast, expressing the CML oncogenic translocation product *BCR–ABL* in the same progenitor cells failed to induce myeloproliferative disease (4). Therefore, it seems that in CML, normal HSC is the disease cell of origin, whereas in AML, committed progenitor cells can regain self-renewal ability through oncogenic events and become LSCs. Another remaining question is whether the cell of origin is determined by the initial oncogenic event. The study by Huntly and colleagues (4) suggested that different oncogenic events have various degrees of transformation capabilities, and such differences may explain the different cells of origin in different leukemias. In a mouse model of *MLL–AF9* leukemia, the dosage of MLL–AF9 expression could affect the transformation susceptibility of different cell types. Progenitor cells, such as GMPs, could be transformed only when a very high dose of MLL–AF9 was expressed (5). Hence, understanding the initiating event is as important as the identification of cancer's cell of origin. A third unresolved question is: What is the sequence of events that leads to the clonal evolution from a normal HSC/progenitor to a preleukemic cell and eventually leukemia? According to the Nowell model, LSCs would acquire additional genetic abnormalities in a stepwise fashion that facilitate the progress of disease development and subsequent relapses. Indeed, whole-exome sequencing (WES) and whole-genome sequencing of more than 200 adult *de novo* AMLs have discovered that, on average, each AML genome carries 13 mutations located within genes (6), among which only five are recurrently mutated in AML. However, sequencing studies on bulk tumors can only infer clonal evolution based on the mutation allele frequency and could not provide information such as at which exact stage along the LSC differentiation these mutations are acquired. Moreover, biologic experiments are needed to differentiate

[1]Institute for Computational Biomedicine, Weill Cornell Medical College, New York, New York. [2]Department of Medicine, Weill Cornell Medical College, New York, New York. [3]Institute for Precision Medicine, Weill Cornell Medical College, New York, New York. [4]Sandra and Edward Meyer Cancer Center, Weill Cornell Medical College, New York, New York.

Corresponding Author: Olivier Elemento, Weill Cornell Medical College, 1305 York Avenue, New York, NY 10021. Phone: 646-962-5726; Fax: 646-962-0383; E-mail: ole2001@med.cornell.edu

doi: 10.1158/2159-8290.CD-14-0743

driver mutations from passenger mutations that have no contribution to leukemogenesis. Without such knowledge, it is difficult to develop targeted therapies to eradicate LSCs.

In this issue of *Cancer Discovery*, Damm and colleagues (7) sought to address these important questions in the context of chronic lymphocytic leukemia (CLL). CLL is the most common adult leukemia in the Western world. This disease is characterized by the clonal expansion of $CD5^+CD23^+$ B cells in blood, bone marrow, and secondary lymphoid tissues. Roughly half of the patients with CLL carry mutations within the immunoglobulin heavy-chain variable-region genes (*IGHV*), which is frequently associated with an indolent disease. Patients with unmutated *IGHV*s often present with more aggressive disease. Previously, xenotransplantation studies have demonstrated that HSCs isolated from patients with CLL were primed toward lymphoid lineage and were prone to develop features of CLL, suggesting that normal HSCs may be the cell of origin of CLL (8). In this study, the authors used cell-surface markers to isolate populations of immature progenitor cells ($CD34^+$) and mature T cells ($CD3^+$), monocytes ($CD14^+$), and normal and tumor B cells ($CD19^+$) from 24 patients with CLL, and then surveyed the mutational landscapes in these cell populations by WES. Strikingly, they observed that in 21 of 24 patients, a subset of the CLL mutations seen in the $CD19^+$ B cells could be detected in either or both the immature $CD34^+$ cells and the $CD14^+$ monocytes in the myeloid lineage from the same patient, suggesting that CLL pathogenesis involves immature progenitor cells (7). Moreover, the authors sorted $CD34^+CD19^-$ progenitor cells from 18 patient samples and cultured them in myeloid conditions. These cells yielded myeloid colonies, of which colonies from 13 patients had detectable CLL mutations, demonstrating that these CLL mutation–carrying immature progenitor cells still had multilineage potential. Mutations detected in the progenitors of CLL patients targeted several well-known CLL oncogenes, including *NOTCH1*, *SF3B1*, *TP53*, *XPO1*, *BRAF*, and *MLL2*. In addition, Damm and colleagues (7) also found mutations in *NFKBIE* and *EGR2* in the progenitor cells of several patients with CLL. Of note is that those patients with *EGR2* mutations were associated with higher $CD38^+$ expression (a poor prognostic marker), shorter time to re-treatment, and low 5-year overall survival, further suggesting that mutations in *EGR2* may be a potential initiating event in progenitor cells, resulting in preleukemic cells that would eventually develop into CLL. Indeed, when these authors expressed EGR2 mutants in a murine multipotent hematopoietic cell line, EML, they observed that these cells exhibited slower growth and faster loss of the $B220^+$ and $Gr1^+$ population than EML cells transduced with wild-type EGR2. How these changes affect the development of CLL requires further investigation, potentially using a xenotransplantation model. Finally, using RNAseq, they identified a gene expression signature in CLL patients with *EGR2* mutations. In particular, the upregulated signature of *EGR2*-mutated CLL samples was enriched of genes induced by B-cell receptor (BCR) stimulation in normal B cells, suggesting a deregulation of BCR signaling in these patients (7).

Taken together, results from this study confirm that the cell of origin of CLL is among the hematopoietic progenitor cells that still have the potential to differentiate into different lineages of mature blood cells, and identify early mutation events in these progenitor cells that may lead to the subsequent transformation of CLL. Recently, similar studies also uncovered preleukemic HSCs of AML and the founding mutations associated with these cells, including *ASXL1*, *DNMT3A*, *IDH2*, and *IKZF1* (9, 10). In addition, Shlush and colleagues (10) elegantly showed that the initiating *DNMT3A* mutation resulted in an expanded pool of HSCs and downstream progenitors, within which additional mutations such as NPM1c were acquired, probably at the GMP and/or multilymphoid progenitor stages, to drive progression to AML. Collectively, these studies raise several important issues in the treatment of leukemias. First, in addition to the eradication of leukemia blasts by conventional chemotherapy, new effective therapies are needed to selectively kill preleukemic HSCs and progenitor cells that are the real source of leukemias. Although this remains a major clinical challenge, the newly discovered features of these rare cell populations, that is, upregulated BCR signaling gene expression in *EGR2*-mutated CLLs and expanded HSC pools in *DNMT3A*-mutated AMLs, provide opportunities for the development of innovative targeted therapy in the near future. Second, the detection of minimal residual disease after initial treatment and the monitoring of relapse disease development need to be redesigned to trace these preleukemic cells that often escape and survive chemotherapy. Multicolor flow sorting and ultrasensitive digital PCR may need to be adopted in the clinical setting to detect rare preleukemic cells in peripheral blood samples routinely. Third, in this personalized precision-medicine era, for those patients with leukemia without a known oncodriver mutation, it may be beneficial to include xenotransplantation animal models to pinpoint the disease-driving mutation in the preleukemic stem cells.

Looking back to the Nowell model, it is amazing how accurate his prediction of the clonal evolution course of cancer was. Thirty years later, we finally have the right tools and assays to fully address the question of cancer's cell of origin. It is foreseeable that an explosion of research on this topic will occur in the next several years and significantly advance our understanding of cancer and improve how we treat this devastating disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online September 2, 2014.

REFERENCES

1. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23–8.
2. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645–8.
3. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730–7.
4. Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic

stem cells to committed murine hematopoietic progenitors. Cancer Cell 2004;6:587–96.
5. Chen W, Kumar AR, Hudson WA, Li Q, Wu B, Staggs RA, et al. Malignant transformation initiated by Mll-AF9: gene dosage and critical target cells. Cancer Cell 2008;13:432–40.
6. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059–74.
7. Damm F, Mylonas E, Cosson A, Yoshida K, Della Valle V, Mouly E, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. Cancer Discov 2014;4:1088–1101.
8. Kikushige Y, Ishikawa F, Miyamoto T, Shima T, Urata S, Yoshimoto G, et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell 2011;20:246–59.
9. Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A 2014;111:2548–53.
10. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 2014;506:328–33.

Tracing the Roots of Cancer Evolution

Yanwen Jiang and Olivier Elemento

Cancer Discovery 2014;4:995-997.