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Tracing the roots of cancer evolution

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Summary

By comparing the genomes of progenitor cells and mature cells of lymphoid and myeloid lineages in CLL patients, Damm and colleagues confirmed that CLL originates from pre-leukemic CD34+ progenitor cells and identified early CLL mutations that are associated with these progenitor cells. Moreover, they discovered that deregulation of BCR signaling may be one of the hallmarks of CLL, particularly in tumors with EGR2 mutations.

What is the cell of origin of tumors? This fundamental question has challenged cancer research for decades. Almost thirty years ago, in a review published in *Science*, Peter C. Nowell 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” (1). This ground-breaking theory, now known as the cancer clonal evolution model, was partly based on the observation he made with David Hungerford that in chronic myelogenous leukemia (CML) patients, essentially all tumor cells carried a chromosomal translocation (*BCR-ABL*) known as the Philadelphia chromosome. 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.

Hematological 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 multi-color 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).

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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 AML patients. Evidence from animal experiments has supported both models. For example, Huntly et al. demonstrated that expression of oncogenic AML fusion protein MOZ-TIF2 in common myeloid progenitors (CMP) and granulocyte-monocyte progenitors (GMP) can recapitulate AML in mice (4). In contrast, expressing the CML oncogenic translocation *BCR-ABL* in the same progenitor cells failed to induce myeloproliferative disease (4). Therefore, it appears that in CML, normal HSC is the disease cell of origin; whereas in AML, committed progenitor cells can re-gain self-renewal ability through oncogenic events and become LSC. Another remaining question is whether the cell of origin is determined by the initial oncogenic event. The study of Huntly et al. suggested that different oncogenic events have various degrees of transformation capabilities and such differences may explain the different cell of origins 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 only be transformed when 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 pre-leukemic cell and eventually leukemia? According to Nowell's model, LSC would acquire additional genetic abnormalities in a step-wise fashion that facilitate the progress of disease development and subsequent relapses. Indeed, whole exome (WES) and whole genome sequencing (WGS) of more than 200 adult *de novo* AML have discovered that on average each AML genome carries 13 mutations located within genes (6), among which only 5 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, biological experiments are needed to differentiate 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 et al. sought to address these important questions in the context of chronic lymphocytic leukemia (CLL) (7). 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 CLL patients carry mutations within the immunoglobulin heavy-chain variable-region genes (IGHVs), which is frequently associated with an indolent disease. Patients with unmutated IGHVs often present with more aggressive disease. Previously, xenotransplantation studies have demonstrated that HSCs isolated from CLL patients were primed towards lymphoid lineage and were prone to develop features of CLL, suggesting normal HSCs may be the cell of origin of CLL (8). In this current 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 CLL patients, and then surveyed the mutational landscapes in these cell populations by WES. Strikingly, they observed that in 21 out 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 multi-lineage potentials. 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 *et al* also found mutations in *NFKBIE* and *EGR2* in the progenitor cells of several CLL patients (7). Of note is that those patients with *EGR2* mutations were associated with higher CD38+ expression (a poor prognosis marker), shorter time to retreatment and low 5-year overall survival, further suggesting that mutations in *EGR2* may be a potential initiating event in progenitor cells resulting in pre-leukemic 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 B220+ and Gr1+ population than EML cells transduced with wide type *EGR2*. How these changes impact the development of CLL requires further investigation, potentially using a xenotransplantation model. Finally, using RNAseq, they identified a gene expression signature of CLL patients with *EGR2* mutations. In particular, the upregulated signature of *EGR2*-mutated CLL samples was enriched of genes induced by 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 identified early mutation events in these progenitor cells that may lead to the subsequent transformation of CLL. Recently, similar studies also uncovered pre-leukemic HSCs of AML and the founding mutations associated with these cells, including *ASXL1*, *DNMT3A*, *IDH2*, and *IKZF1* (9, 10). In addition, Shlush *et al.* elegantly showed that the initiating *DNMT3A* mutation resulted in an expanded pool of HSCs and downstream progenitors, within which additional mutations such as those in *NPM1c* were acquired, probably at the GMP and/or MLP stages, to drive progression to AML (10). Collectively, these studies raised 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 pre-leukemic 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, i.e. upregulated BCR signaling gene expression in *EGR2*-mutated CLLs and expanded HSC pools in *DNMT3A*-mutated AMLs, provide opportunities for 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 re-designed to trace these pre-leukemic cells that often escape and survive chemotherapy. Multi-color flow sorting and

ultra-sensitive digital PCR may need to be adopted in clinical setting to detect rare pre-leukemic cells in peripheral blood samples routinely. Third, in this personalized precision medicine era, for those leukemia patients without a known onco-driver mutation, it may be beneficial to include xenotransplantation animal models to pinpoint the disease driving mutation in the pre-leukemic stem cells.

Looking back to Nowell's 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.

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