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Surprise! HSC Are Aberrant in Chronic Lymphocytic Leukemia

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In this issue of *Cancer Cell*, Kikushige et al. report the surprising finding that, in human chronic lymphocytic leukemia (CLL), hematopoietic stem cells (HSC) aberrantly generate clonal B cells with CLL-like phenotypes, which implicate HSC in the pathogenesis of this mature lymphoid malignancy and has major implications for CLL therapies.

Blood represents arguably the most dynamic of human tissues, orchestrating not only its own complex homeostasis, but that of virtually every physiological system. Within this dynamic tissue, normal hematopoiesis is organized as a cellular hierarchy initiated and maintained by self-renewing hematopoietic stem cells (HSC) that give rise to lineage-restricted progenitors, which eventually produce all the mature cells of the blood. Cancers can develop from any of these blood lineages, resulting in many different diseases including myeloproliferative disorders, acute and chronic leukemias, lymphomas, and myeloma.

While several myeloproliferative disorders are known to arise at the HSC stage in hematopoiesis, most other hematologic malignancies have been linked to later stages in this hierarchy. For example, aggressive acute leukemias are generally believed to arise from early progenitors, with some initial leukemogenic events occurring in HSC themselves. Further, the most common class of hematopoietic malignancies, the mature B cell lymphomas and leukemias, are classified according to their presumed cell of origin along the continuum of B cell development and differentiation. For these B cell tumors, relationship to the germinal center (GC) reaction within secondary lymphoid organs is central for their classification as pre-GC, GC, or post-GC neoplasms, as these "mature" tumors are believed to arise from B cells, following VDJ recombination at immunoglobulin loci and independent of early hematopoiesis and HSC. This consideration has a major effect on therapeutic targeting, with agents selected for activity against mature B cells.

Much of the evidence supporting a cell-of-origin model linking mature B cell tumors to post-VDJ recombination stages of lymphoid ontogeny, and with the germinal center reaction, has been indirectly based on shared phenotypes, including cell surface markers and capacity for ongoing somatic hypermutation (Küppers, 2005). Nonetheless, direct evidence for this model comes from the presence of identical (or nearly identical) VDJ recombination events at the IGH locus in paired samples from: (1) donors and recipients involved in inadvertent transmission of lymphoid malignancies during allogeneic hematopoietic cell transplantation, (2) samples at diagnosis and relapse of mature B cell tumors, (3) samples before and after histological transformation from indolent to aggressive lymphomas, and (4) composite lymphomas occurring within individual patients with, for example, both Hodgkin's disease and a non-Hodgkin's lymphoma (Martinez-Climent et al., 2010).

This view of mature B cell malignancies is challenged by the findings reported in this issue by Kikushige et al. (2011) through their investigation of human chronic lymphocytic leukemia (CLL). CLL is a mature B cell malignancy that is typically characterized by the accumulation of clonal mature B cells in the bone marrow, blood, and lymph nodes that aberrantly express CD5. In nearly all cases, CLL is preceded by a preleukemic monoclonal B cell lymphocytosis (MBL) (Landgren et al., 2009), though only a subset of patients with MBL are prone to develop CLL over their lifetime (Rawstron et al., 2008). While the precise identity of the normal counterparts of CLL and MBL remains unclear, most studies suggest transformation of cells after VDJ recombination (Chiorazzi and Ferrarini, 2011). In current standard therapy, CLL is treated with chemotherapeutics active against B lymphocytes, including DNA-alkylating agents and nucleoside inhibitors such as cyclophosphamide, bendamustine, fludarabine, and pentostatin. In many cases, CLL is also treated with monoclonal antibodies including rituximab and ofatumumab, which target CD20 on the surface of mature B cells (Chiorazzi et al., 2005).

Strikingly, nearly identical VDJ-recombination events can rarely be observed as independent events in different patients with CLL and MBL, which can even include stereotyped or quasi-identical sequences in the nongermline-encoded portion (HCDR3) of rearranged immunoglobulin genes (Messmer et al., 2004). Additionally, the usage of immunoglobulin variable genes is known to be biased within the B cell receptors of mature lymphoid tumors, particularly within CLL and MBL. These observations suggest the presence of selective pressures that favor specific VDJ clones, including intrinsic factors such as heritability of MBL and CLL as well as extrinsic ones such as undefined autoantigen(s) or pathogen(s) capable of stimulating and promoting such B cell clones.

In the current study, Kikushige et al. (2011) investigate the cellular origin of CLL by isolating subpopulations and assaying their ability to engraft disease upon xenotransplantation into immunodeficient

mice. Consistent with prior studies, neither the CD19+ B cells, nor the CD34+CD38+CD10+CD19+ pro-B cells stably engrafted in vivo. The CD34+CD38-(CD90+) fraction, known to be highly enriched for HSC in normal marrow, engrafted stably and gave rise to bilineage lymphoid-myeloid hematopoiesis. Remarkably, these engrafted cells exhibited an increased proportion of polyclonal pro-B cells, and analysis of VDJ recombinations in the downstream B cells revealed mono- or oligoclonality in a population with features of MBL, including CD5 expression. Further examination of the VDJ recombination events determined that they were different from those present in the CLL cells. Nonetheless, as in human CLL and MBL, a biased V-gene repertoire was observed in the lymphoproliferative disorder seen in these mice, which also shared the tendency for IgVH somatic mutation with the corresponding CLL. Moreover, the engrafted cells showed no evidence of genomic abnormalities present in the original cancer.

How can all this be put together? It appears that HSC in CLL are involved in disease pathogenesis, serving as aberrant preleukemic cells that produce an increased number of polyclonal pro-B cells. The resulting mature B cells are selected, likely by autoantigen(s), resulting in mono- or oligoclonal B cell populations. These clones differ from those present in the corresponding CLL, perhaps due to selection by different autoantigen(s) present in the human, but not the mouse, host. But this is not frank CLL, instead modeling MBL. At some point along the differentiation pathway, additional genomic abnormalities develop, eventually resulting in the clonal CLL disorder.

If this model reflects the biology of the in situ human disease, one would expect to detect oligoclonal disease in CLL and/or MBL patients. Indeed, oligoclonality of VDJ recombination events is common in MBL and is also seen in 10%–20% of CLL cases. However, this model fails to account for the fact that CLL patients who achieve molecular remissions, but ultimately recur, do not relapse with polyclonal disease or distinct clones.

The finding of aberrant HSC in CLL raises the question of what mechanisms drive this process. Presumably, genetic and/or epigenetic mutations are present in these cells and determine their aberrant behavior. Recently, next generation sequencing identified recurrent somatic mutations, presumably driving transformation, in CLL (Puente et al., 2011). It will be of great interest to determine if these mutations are present in HSC from CLL patients.

Another implication of these results is that B cell antigen receptor (BCR) signaling is central to the pathogenesis of CLL, resulting in the production of mono- or oligoclonal B cells from polyclonal pro-B cells. Once the CLL clone is established, it is uncertain if continued signaling is required for the disease. However, recently, chronic active BCR signaling has been implicated in the pathogenesis of a subtype of diffuse large B cell lymphoma (Davis et al., 2010). Consistent with this, inhibitors of BCR signaling show activity in several lymphoproliferative disorders including CLL.

Nearly all current therapies for CLL, including rituximab, target the mature B cell leukemic population; however, it may be that therapies effective at targeting these CLL cells will not affect the preleukemic HSC. Thus, curative strategies may need to target not only the frankly leukemic cells, but also the preleukemic HSC. How to preferentially target these preleukemic HSC, as compared to residual normal HSC, represents a major challenge that will depend on investigations into the mechanisms that give rise to these aberrant cells.

REFERENCES

Chiorazzi, N., and Ferrarini, M. (2011). Blood *117*, 1781–1791.

Chiorazzi, N., Rai, K.R., and Ferrarini, M. (2005). N. Engl. J. Med. *352*, 804–815.

Davis, R.E., Ngo, V.N., Lenz, G., Tolar, P., Young, R.M., Romesser, P.B., Kohlhammer, H., Lamy, L., Zhao, H., Yang, Y., et al. (2010). Nature *463*, 88–92.

Kikushige, Y., Ishikawa, F., Miyamoto, T., Shima, T., Urata, T., Yoshimoto, G., Mori, Y., Iino, T., Yamauchi, T., Eto, T., et al. (2011). Cancer Cell *20*, this issue, 246–259.

Küppers, R. (2005). Nat. Rev. Cancer *5*, 251–262.

Landgren, O., Albitar, M., Ma, W., Abbasi, F., Hayes, R.B., Ghia, P., Marti, G.E., and Caporaso, N.E. (2009). N. Engl. J. Med. *360*, 659–667.

Martinez-Climent, J.A., Fontan, L., Gascoyne, R.D., Siebert, R., and Prosper, F. (2010). Haematologica *95*, 293–302.

Messmer, B.T., Albesiano, E., Efremov, D.G., Ghiotto, F., Allen, S.L., Kolitz, J., Foa, R., Damle, R.N., Fais, F., Messmer, D., et al. (2004). J. Exp. Med. *200*, 519–525.

Puente, X.S., Pinyol, M., Quesada, V., Conde, L., Ordóñez, G.R., Villamor, N., Escaramis, G., Jares, P., Beà, S., González-Díaz, M., et al. (2011). Nature *475*, 101–105.

Rawstron, A.C., Bennett, F.L., O'Connor, S.J.M., Kwok, M., Fenton, J.A.L., Plummer, M., de Tute, R., Owen, R.G., Richards, S.J., Jack, A.S., and Hillmen, P. (2008). N. Engl. J. Med. *359*, 575–583.