Chronic lymphocytic leukemia (CLL) is a biologically heterogeneous B-cell neoplasm that can be stratified into two major clinical and molecular subtypes according to the mutational status of the immunoglobulin heavy chain variable (IGHV) region. Approximately half of all CLL cases carry unmutated IGHV genes (uCLL), while the remaining half has somatically mutated IGHV genes (mCLL; ref. 1). Monoclonal B-cell lymphocytosis (MBL) is a tiny expansion of phenotypically clonal B cells that shares the same immunophenotype of overt CLL and temporally precedes it (2). MBL is a premalignant condition that is occasionally discovered in otherwise healthy subjects and can be categorized as either low count or high count based on the size of the clone. While high-count MBL has the same incidence of CLL, low-count MBL is 100-fold more frequent and can be detected in approximately 10% of adults over the age of 40 years (2). Many of the biological characteristics of high-count MBL are similar to those of early-stage CLL, indicating that they are the same disease. Indeed, the $5 \times 10^9$/L B-cell count threshold that distinguishes high-count MBL from CLL was initially selected on analytic, but not biological, grounds and then maintained afterward to allow homogeneous reporting of clinical trial cohorts. Conversely, low-count MBL does not match with either high-count MBL or CLL at the immunogenetic and molecular levels (2). Collectively, these findings, along with observational studies, point to a stepwise evolution from high-count MBL to CLL but indicate that relatively few individuals with low-count MBL progress to high-count MBL or CLL.

In this issue of Blood Cancer Discovery, Kretzmer and colleagues (3) show that the aberrant cancer methylome of CLL is already established at the precursor MBL stage. The CLL methylome is then maintained unchanged during the course of the disease for years, irrespective of the selective pressure imposed to the leukemic cells by chemoimmunotherapy or pathway inhibitors (Fig. 1). They clearly demonstrate that the switch to an abnormal DNA methylation landscape consistently occurs before the MBL landmark. They also show that aberrant DNA methylation homogeneously affects single cells. Hence, the transition to the altered methylome occurs very early, and once acquired, it is a trunk and extremely stable change. However, the precise time point when the leukemic clone starts deviating significantly from the normal differentiation trajectory is still elusive, although the process should be relatively fast.

DNA methylation, the most common chemical DNA modification, provides information on different aspects of a tumor, including the cell of origin, the pathways that are relevant for normal-to-cancer transition, and the age of the transformed clone. DNA methylation, which is inversely proportional to cognate gene transcription, is attenuated during cell differentiation in gene regulatory elements involved in cell specification and is accompanied by a global gain of DNA methylation in pluripotency-specific genes, resulting in cell types with different methylomic profiles (4). Therefore, DNA methylation physiologically defines cellular identity. The DNA methylation fingerprint of a cell that has acquired a cancer transforming hit is “frozen” at its differentiation stage and stably propagated in the daughter tumor cells. Accordingly, the DNA methylome is reminiscent of the cell of origin of the tumor (4).

The exact cell of origin of CLL is still debated. IGHV mutations are acquired during antigen-based affinity maturation of activated B cells in the germinal center undergoing somatic hypermutation (SHM) after antigen recognition. Hence, the IGHV mutation status of a B-cell tumor is a proxy of the developmental stage of its cell of origin with respect to the germinal center landmark. According to this model, uCLL arises from B cells that matured independently of the germinal center reaction and that were not touched by the SHM process. Conversely, mCLL originates from B cells that underwent SHM after antigen recognition and while transiting through the germinal center (5). The immunogenetic-derived paradigm of the CLL cell of origin is complemented by comparative unbiased phenotypic profiling of CLL versus normal B cells at diverse differentiation stages, which indicate that both mCLL and
uCLL are similar to antigen-experienced B cells, with mCLL and uCLL subtypes having been derived from postgerminal center or germinal center–independent cells, respectively (6).

In the DNA methylation–driven phylogenetic tree model of Kretzmer and colleagues (3), uCLL and mCLL stem from the same node, and their branches are developmentally closer to the branch of memory B cells than to the branch of naïve B cells. This observation is consistent with previous findings that CLL by methylation status reflects the postgerminal center memory B-cell epigenome (7).

Transforming events acquired during tumor initiation are associated with further alterations of the DNA methylation state that occur in the tumor-initiating cell. Therefore, the resulting tumor DNA methylation pattern is a composite of the cell of origin–specific plus tumor-specific events. Kretzmer and colleagues (3) describe a set of chromatin regions whose DNA methylation state emerges from the background imprint of the cell of origin. These de novo differentially methylated regions of the genome are already detectable at the preleukemic MBL stage, suggesting that they are involved in early premalignant steps of the disease. Kretzmer and colleagues (3) also show that CLL acquires more tumor-specific DNA hypomethylation than hypermethylation. Tumor-specific DNA hypomethylation is enriched at the binding sites of transcription factors that are expressed in CLL and have a previously reported association with its pathogenesis, such as NFATC1 (7, 8), a known downstream target of active signaling from the B-cell receptor (BCR). In CLL cells, BCR signaling is chronically activated and involved in the maintenance and expansion of the CLL cell clone.

Whether CLL is addicted to de novo DNA methylation changes is an unanswered question. Altered DNA methylation is a vulnerability of myeloid malignancies, which harbor recurrent mutations in genes governing DNA methylation (e.g., TET2, DNMT3A, and IDH1/IDH2) and are highly sensitive to DNA methyltransferase inhibitors (9). This is not the case for CLL.

The search for de novo differentially methylated regions requires inferring the physiologic DNA methylation dynamics occurring during normal B-cell differentiation, modeling the putative cell of origin, and then subtracting this physiologic background from the DNA methylation signals identified in CLL samples. B-cell subsets utilized in DNA methylation studies are discrete snapshots of a continuum of different cell specifications and may not reflect the very precise B-cell differentiation stage of the CLL cell of origin. Hence, what is now termed as tumor-specific DNA methylation change in CLL might just reflect the imprinting of the yet unknown cell of origin. Coupling DNA methylation profiling to single-cell technologies, as pivotally done by Kretzmer and colleagues (3), may better resolve epigenome changes unfolding during mature B-cell differentiation, where heterogeneous cell populations exist in a continuum.

The DNA methylation state is reestablished after each cell replication, but errors occur in the fidelity of this process. Therefore, changes in the DNA methylation profile in certain
regions of the genome surrogate the count of cell-specific mitoses; mark the proliferative history of a cell; and allow, when assessed at the single-cell level, the reconstruction of the DNA methylation–based evolutionary tree of the tumor and its relative proliferation history. This knowledge has led to the concept of using DNA methylation as a mitotic clock (8, 10).

At the single-cell level, DNA methylation–driven phylogenetic trees of CLL exhibit early branching with homogeneous branching from the tree trunk, consistent with a rapid drift from the normal counterpart after the initial malignant transformation (10). CLL trees are longer than any normal B-cell subpopulation tree, indicating an expected excess of replications in leukemic cells compared with normal cells. As expected, naïve B cells are younger than memory B cells according to the DNA methylation–based mitotic clock (10). The observation by Kretzmer and colleagues (3) that uCLL and mCLL are older than pregerminal center–naïve B cells and have a similar epigenetic age as postgerminal center memory B cells points toward an antigen-experienced, “memory-like” B lymphocyte evolved through germinal center–dependent (mCLL) or germinal center–independent (uCLL) trajectories as the cell of origin of CLL.

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REFERENCES

Epigenetic Trajectories of the Premalignant-to-Malignant Transition of Chronic Lymphocytic Leukemia

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