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Prevalent premalignancy

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The presence of hematologic malignancy–associated mutations in the blood of individuals without cytopenias or dysplasia has been termed clonal hematopoiesis of indeterminate potential (CHIP), a clinical entity associated with increased age, risk of developing hematologic malignancy, and decreased overall survival.[1] Two papers in this issue of *Blood* take different approaches to advance our understanding of the frequency and clinical characteristics of clonal hematopoiesis. Zink et al analyze whole genome sequencing data from the blood of >11 000 healthy Icelandic individuals, and Buscarlet et al perform targeted sequencing on the blood of 2530 healthy older women.[2,3]

The presence of clonal hematopoiesis was first inferred from the finding that X-chromosome inactivation, predicted to be a purely stochastic event, is often skewed in the blood of otherwise healthy women, a subset of whom were later shown to harbor mutations in the *TET2* gene.[4] In addition, hematopoietic stem cells (HSCs) carrying preleukemic mutations can be detected in both leukemia and remission samples obtained from patients.[4] More recently, the presence of somatic variants in the blood of healthy individuals has been used to identify and characterize clonal hematopoiesis. Analysis of exome sequencing data from >30 000 people identified recurrent somatic hematologic malignancy–associated mutations in up to 10% of patients over the age of 65.[5-7] Together, these findings have led to a model in which HSCs acquire mutations over time leading to clonal expansion and eventually to overt malignancy.[8]

Most somatic mutations are biologically silent passenger events that create a mutational signature marking each HSC and its progeny.[9] The presence of a detectable collection of somatic mutations within the blood is therefore suggestive of clonal expansion from a single HSC. Zink et al take advantage of a passenger mutation analysis to develop an unbiased statistical method to measure outliers based on the number of detectable somatic mutations in order to identify clonal hematopoiesis without depending on the presence of known driver mutations. They find the prevalence of clonal hematopoiesis to be almost double that previously reported within each age group, rising to >50% of those >85 years old. A candidate hematologic malignancy–associated driver mutation could only be identified in ~40% of these cases. Clonal hematopoiesis, with or without a candidate driver mutation, was associated with increased risk of hematologic malignancy and death. One caveat to their analytic approach is that not all individuals with a detectable somatic driver mutation had enough passenger mutations to meet mutational outlier criteria for clonal hematopoiesis.

Buscarlet et al measure clonal hematopoiesis using a targeted sequencing panel of 19 genes recurrently mutated in myeloid disease. The increased sequencing depth associated with a targeted approach likely explains the increased prevalence of CHIP (16%) seen in their cohort. The use of ultradeep, error-corrected sequencing has previously demonstrated mutations within *DNMT3A* or *TET2* in up to 95% of older adults, suggesting that if you sequence deeply enough you can find a mutant clone in virtually all subjects.[10] The most common recurrently mutated genes identified by Buscarlet et al are also *DNMT3A* and *TET2*, and these 2 genes account for a somewhat larger proportion (93%) of all mutations than in previous reports.[6,7] They further assess the relationship between these mutations and a variety of hematological parameters, demonstrating no significant correlation between mutation status and any abnormality in the blood. This cohort did not have long-term outcome data available for survival analysis.

The high frequency of clonal hematopoiesis observed by Zink et al in the absence of known driver mutations could be explained by the presence of additional undiscovered driver genes or, alternately, by epigenetic alterations leading to clonal expansion in the absence of a somatic driver mutation. The authors do report 1 new candidate driver gene, metastasis-associated protein 2 (*MTA2*), which encodes a subunit of the NuRD (nucleosome remodeling and histone deacetylase) complex, an epigenetic regulator. Mutations in *MTA2* have previously been found in preleukemic HSCs, and many other epigenetic regulators are recurrently mutated in CHIP.[8,11] Further work will be needed to confirm whether mutations in *MTA2* can, in fact, lead to clonal hematopoiesis and whether there are additional genes that lead to clonal expansion and hematologic malignancy when mutated.

These 2 reports provide evidence of familial predisposition to clonal hematopoiesis. Zink et al note an association between short telomeres and clonal hematopoiesis. They go on to identify a small germ line deletion within the telomerase reverse transcriptase (*TERT*) intron 3 that is associated with an increased risk of development of clonal hematopoiesis. How this deletion might alter TERT function or lead to increased clonal hematopoiesis is unclear. Because the patient cohort analyzed by Buscarlet et al includes a large number of sibling pairs, they were able to show familial aggregation for *TET2*-mutation acquisition. A similar familial association has been described in patients with myeloproliferative neoplasms.[12]

Another question addressed by Zink et al is whether clonal hematopoiesis is always a consequence of a neoplastic driver mutation or whether clonality might, at times, reflect progressive oligoclonal skewing of HSCs during normal aging. In very old individuals, the number of HSCs that actively contribute to mature blood production may be limited, leading to oligoclonality.[13] Zink et al develop a computational model to demonstrate that some degree of clonality may, in fact, be an inevitable consequence of neutral drift within the HSC compartment over time. Although clonal hematopoiesis in the presence of a known driver mutation (CHIP) has increased risk of malignant transformation and death, it is unclear whether clonal hematopoiesis due to neutral drift has the same pathological consequences.

Multiple reports have now established that CHIP is a distinct clinical entity defined by a common set of mutations with significant risk for progression to hematologic malignancy and death. Collectively, these 2 papers demonstrate that clonal hematopoiesis is more common

than previously reported, may have a genetic predisposition, and is often not associated with an identifiable somatic driver mutation. Future studies are now needed to determine how best to identify patients with clonal hematopoiesis, whether all patients hold the same risk of transformation and death, and how to manage those patients at highest risk.

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● ● ● LYMPHOID NEOPLASIA

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Let's give BACH2 a breath of fresh air

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In this issue of *Blood*, Zhang and colleagues identify that BACH2 acts as a tumor suppressor protein in mantle cell lymphoma (MCL). Low levels of BACH2 are associated with poor outcome in MCL patients and in vitro resistance in cell lines to both targeted agents (proteasome inhibitor bortezomib, Bruton tyrosine kinase inhibitor ibrutinib) and chemotherapy agents (etoposide, methotrexate). BACH2 is negatively regulated in hypoxic conditions, as in the bone marrow microenvironment, via a regulatory loop involving hypoxia-induced factor-1α, heme, and prolyl hydroxylase 3.[1]

BACH2 is a transcription factor expressed in B cells from the pro-B cells to mature B cells and is downregulated during the maturation to plasma cells.[2,3] At the common lymphoid progenitor stage, BACH2 represses the myeloid program, pushing cells toward the B-cell development.[2] At the pre–B-cell stage, BACH2 competes with BCL6 for binding to promoters of genes coding for checkpoint regulators such as TP53 or CDKN2A, causing the negative selection of pre–B cells not bearing functional VDJ rearrangements.[4] At later maturation stages, BACH2 cooperates with BCL6, blocking the differentiation to plasma cells, suppressing BLIMP1 expression, and allowing the somatic hypermutation and class switch recombination to take place.[5] Because of its important role at different maturation stages, it is not surprising that BACH2 is deregulated in neoplastic conditions. The *BACH2* gene locus is recurrently deleted in B-cell tumors,[4] and, similarly to what is reported now in MCL, patients with low BACH2 expression in tumor cells have often been found to have a worse outcome than the remaining patients with higher expression.[4,5]

The activity BACH2 in normal cells is tightly regulated at different levels in a very dynamic way (see figure).[5] Based on its pattern of expression across B cells, BACH2 is regulated at the transcription level, and the transcription factor PAX5 is a positive regulator.[2,3] However, there are numerous examples of how BACH2 is regulated at the protein level. BCL6 maintains BACH2 protein stability in diffuse large B-cell lymphoma cells and in germinal center B cells, their normal counterparts.[3] Heme regulates BACH2 at the protein level, both reducing its stability and decreasing its binding to DNA.[5-7] PI3K/AKT/mTOR-mediated phosphorylation of BACH2 leads to a decreased protein activity, changing its cellular localization from the nucleus to the cytoplasm.[5] Finally, the production of reactive oxygen species (ROS), for example, induced by cytotoxic drugs such as etoposide, doxorubicin, or cytarabine, determines nuclear localization and activation of BACH2, necessary for the drug-induced cell death.[8]

Now, Zhang and colleagues have given BACH2 a breath of fresh air (more specifically, oxygen), studying its role in MCL, but their results also highlight the complexity of the mechanism controlling BACH2 activity, a topic certainly requiring further work.

Do the findings in MCL apply to other lymphomas or leukemias as well? For example, based on 2 MCL cell lines, BACH2 is important for the antitumor response to methotrexate, whereas it seems not necessary in 1 Burkitt lymphoma cell line, in which only drugs inducing ROS benefit from high levels of BACH2.[8] What is indeed the relationship, if any, between BACH2, hypoxia, and ROS in MCL because, in other systems, ROS are upregulated in hypoxia. Because the interaction of BACH2 with additional transcription factors is fundamental in determining the effect on the transcriptome, it will be important to assess these cofactors in MCL. SOX11 is a transcription factor acting as an oncogene in MCL, positively regulating PAX5 and silencing BLIMP1.[9] Do BACH2 and SOX11 interact and cooperate? Do the