genes that encode for regulatory RNA-binding proteins. This includes *HNRNPH1*, which was mutated in 10% of cases when both coding and noncoding mutations were considered. HNRNPH1 is involved in regulation of alternative pre-mRNA splicing. *HNRNPH1* itself can be alternatively spliced, and exclusion of its exon 4 is associated with nonsense-mediated decay and lower HNRNPH1 protein levels. Pararajalingam et al noted that the somatic mutations in *HNRNPH1* were actually mostly intronic, peculiarly clustered in regions flanking exon 4. RNA sequencing showed no differences in *HNRNPH1* mRNA levels, but skewing of transcripts toward inclusion of exon 4 in the presence of these intronic mutations was found. Although the precise molecular mechanism still needs to be worked out, the authors showed that these intronic mutations favor inclusion of exon 4, leading to increased levels of HNRNPH1 protein levels. Interestingly, the authors showed that a higher ratio of unspliced-to-spliced exon 4 *HNRNPH1* transcripts ("splicing ratio") significantly correlated with shorter overall survival in patients receiving R-CHOP, while *HNRNPH1* mutation status alone did not.

Trivial but true, good research raises new questions: If *HNRNPH1* splicing ratio correlates with treatment outcome, yet *HNRNPH1* mutations do not, what other alterations, other genes, or other mechanisms do contribute to inclusion of exon 4? Furthermore, what are the downstream effects of this skewed *HNRNPH1* splicing ratio and increased HNRNPH1 protein levels (i) on other splicing events and the overall transcriptional landscape, and (ii) on a functional level? Last but not least, what is the prognostic or predictive impact of these alterations with other treatment regimens and in other lymphomas, and can this be exploited therapeutically?

In summary, Pararajalingam et al tackle the next level of translational research in MCL, including the assessment and functional characterization of the noncoding genome. This is the way to go, and there is more to come.

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LYMPHOID NEOPLASIA

Comment on Roos-Weil et al, page 585

Epigenomics in Waldenström macroglobulinemia

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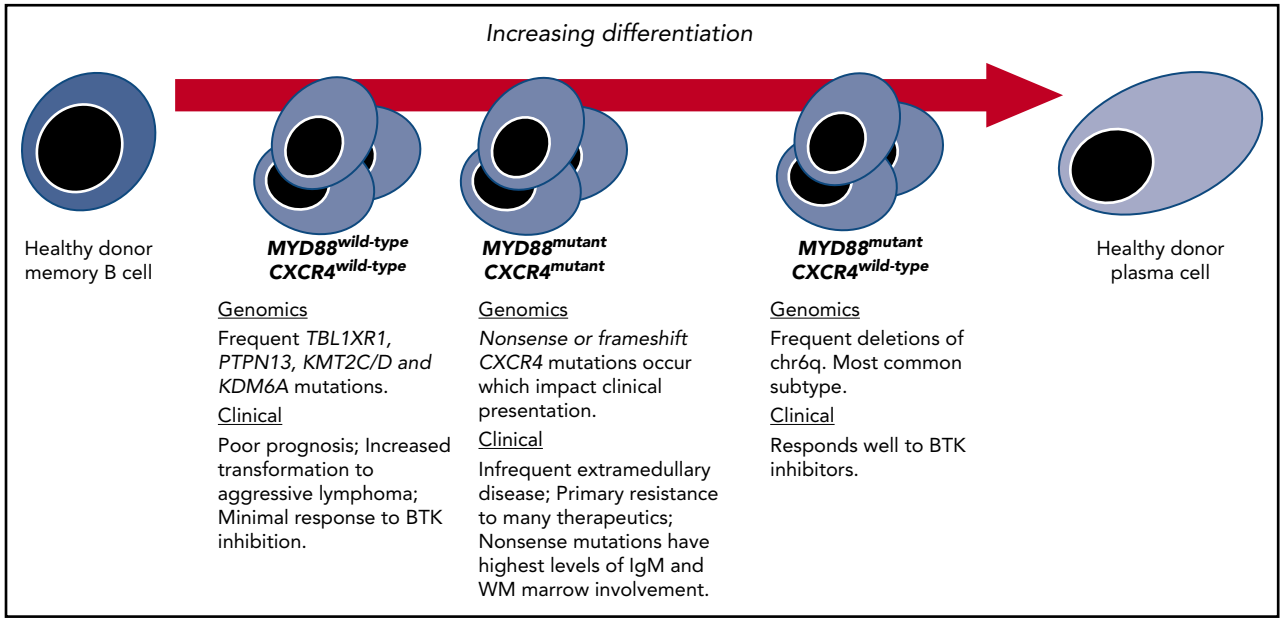
In this issue of *Blood*, Roos-Weil et al[1] identified 2 distinct DNA methylation subtypes for Waldenström macroglobulinemia (WM) by performing methylation screens in 35 well-characterized patients using genome-wide BeadChip technology. In 24 patients, the data were supplemented by transcriptome and targeted DNA sequencing permitting a detailed, multi-omic characterization of WM. Although individual epigenetic events have been previously characterized, this is the first genome-wide characterization and represents an important milestone for understanding WM pathogenesis.

WM is an ideal disease model for a multi-omic approach as it features highly recurring somatic activating mutations, including *MYD88* and *CXCR4* mutations.[2] Moreover, *MYD88* and *CXCR4* activating mutations are clinically relevant as they associate with important disease-presenting features and have prognostic and/or predictive treatment roles.[3] A number of structural alterations have also been identified, the most common being the deletion of chromosome 6q (chr6q) in 30% to 50% of WM patients with mutated *MYD88*, but which are absent in those with wild-type *MYD88*.[2,3] Deletions in chr6q are not unique to WM and are found in other lymphomas, and myeloma, and often occur with chromosome 6p gains. These highly recurring somatic events permit even a modest size multi-omic study such as the one reported by Roos-Weil et al to be informative.

Central to the findings of Roos-Weil et al was the finding that within mutated *MYD88* WM patients, the methylome stratified patients into 2 camps: one with similar profiling to healthy donor (HD) memory B cells (MBC-like) and the other similar to HD plasma cells (PC-like). Those WM patients with MBC-like profiling showed DNA methylation changes



Genetic subtypes and the spectrum of differentiation in WM. Key genomic and clinical characteristics of WM disease based on revelations made possible by integrating transcriptome and epigenomic findings.

that targeted functional domains related to transcriptional activation, whereas among those with PC-like profiling, broader losses in methylation that impacted repressed, heterochromatic, as well as intergenic regions were observed. The notion that certain WM patients have greater plasmacytic differentiation has been long recognized at the morphological and transcriptional level.[4] However, the ability to so clearly define and characterize these groups by differences in methylation offers new and valuable insights, particularly the association of chr6q deletions with a PC-like clone. In previous work, we observed a lack of chr6q deletions in samples from WM patients with wild-type *MYD88* by whole-genome and -exome sequencing and inferred that chr6q deletions represented a likely second hit following acquisition of mutated *MYD88*.[2,5] Other studies speculated that chr6q deletions were critical to the transformation of immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (MGUS) to WM.[6-8] The findings by Roos-Weil et al suggest that such clones, regardless of when and where chr6q deletions occurred, are destined for a PC-like clone.

CXCR4 mutations represent early somatic events that occur soon after the *MYD88* mutation and are detectable even in those individuals with IgM MGUS.[2] Previous transcriptional profiling of samples from patients with both *MYD88* and *CXCR4* mutations showed less plasmacytic differentiation compared with patients who were *MYD88* mutated but wild-type for *CXCR4*.[5] With the data now in hand from Roos-Weil et al, we can assert that *CXCR4* mutations are characteristic of an earlier, MBC-like WM clone, whereas chr6q deletions are characteristic of a more differentiated PC-like clone. *CXCR4* mutations have also been observed in *MYD88* wild-type patients, which is notable as they represent the least differentiated form of WM.[5,6] A number of clinically relevant disease features are associated with *CXCR4* mutations, including lower rates of deep responses, and shorter progression-free survival with treatment using BTK inhibitors.[3] Roos-Weil and colleagues provide an additional dimension challenging us to take into account the importance of the epigenome to such differences in therapeutic outcome.

The data presented by Roos-Weil et al also complement our previous work that examined methylation differences within WM based on mutation status using enhanced reduced representational bisulfite sequencing.[9] As was observed in our RNASeq analysis, pairwise multidimensional scaling of the methylation status of the top 2000 high-variance promoters revealed segregation of samples from patients expressing $MYD88^{mutant}/CXCR4^{wild\text{-}type}$ vs those with $MYD88^{mutant}/CXCR4^{mutant}$ and $MYD88^{wild\text{-}type}/CXCR4^{wild\text{-}type}$. Promoter level analysis revealed 556 differentially methylated promoters in samples from $MYD88^{mutant}/CXCR4^{mutant}$ patients of which 440 (78%) had increased methylation relative to $MYD88^{mutant}/CXCR4^{wild\text{-}type}$ samples. These findings were consistent with greater hypomethylation associated with plasmacytic differentiation. Effected genes included *IL15*, *GNAO1*, *HIF1A*, *SOCS6*, *PIK3R5*, *IRF8*, and *CD38*. For $MYD88^{wild\text{-}type}/CXCR4^{wild\text{-}type}$ samples, and 126 promoters showed significantly different methylation with only 28 (22%) demonstrating increased methylation relative to $MYD88^{mutant}/CXCR4^{wild\text{-}type}$ samples. Effected promoters included *NRIP1*, *FNBP1L*, and *PTK2* in these samples.

Although these observations may tempt thinking of WM as 2 separate diseases based on differentiation status, it is important to consider the crucial role that mutated *MYD88* plays as the primary driver of prosurvival signaling. Indeed, when mutated MYD88 signaling was blocked, signaling through wild-type or mutated CXCR4 failed to rescue cells in the presence of CXCL12, the ligand for the CXCR4. Moreover, although chr6q deletions and *CXCR4* mutations appear mutually exclusive, their gene expression profiles relative to *MYD88* mutant WM patients lacking these events overlap significantly with a set of genes moving in

the same direction with a similar log fold change.[8] As both *CXCR4* mutations and chr6q deletions are thought to be events occurring after the acquisition of *MYD88*, this shared signaling suggests a common path in the evolution to symptomatic WM, and possibly one that might be exploited therapeutically.

Although the findings by Roos-Weil et al provide great insights into the epigenome of WM, they also contribute to our understanding of the topography of the WM genome. The root cause of methylation differences within WM remains to be discerned and represents a critical research question relevant to not only WM pathogenesis but also therapeutic targeting. Transgenic modeling has revealed that methyltransferases such as *DNMT3A/B* impact B-cell activation, plasmacytic differentiation, and humoral immunity.[10] Other contributors worthy of investigation include regulators of histone methylation such as *KMT2A*, *KMT2D*, and *KDM6A* that are frequently mutated in WM.[2]

In summary, Roos-Weil and colleagues have added a third dimension to our understanding of WM genomics, complementing revelations in the transcriptome and genome of WM, and allowing us to demarcate WM based on a spectrum of lymphoplasmacytic differentiation (see figure).

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Aprile et al, page 610

Targeting the bone marrow niche in hemoglobinopathies

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In this issue of *Blood*, Aprile and colleagues demonstrate that in β-thalassemia hematopoietic stem cells (HSCs) are impaired due to defective interaction with the bone marrow microenvironment (BME), a defect that can be rescued by administration of parathyroid hormone (PTH).[1]

This study illuminates the fact that β-thalassemia not only impairs red cell production but also affects global bone marrow (BM) homeostasis, including HSCs and their BM niche, a concept likely relevant to other hemoglobinopathies.

In the genetic disorder β-thalassemia, lack of β-globin synthesis results in peripheral hemolysis, anemia, and ineffective erythropoiesis. The severe form of this disease, β-thalassemia major, requires lifelong transfusions and iron chelation therapy. A major consequence of the disease and treatment is iron overload, which inflicts chronic oxidative organ damage over time, reducing life span.[2] Sickle cell disease is another severe hemoglobinopathy resulting from defective β-globin production. It is characterized by anemia and vasoocclusive crises, which cause ischemic and oxidative organ damage and decrease life span.[2] Despite differences in etiology, the sequelae of altered erythropoiesis in both diseases inflict chronic and systemic injury to all organs, including the BM.

For β-thalassemia major and severe sickle cell disease, hematopoietic cell transplantation (HCT) from an HLA-identical sibling donor is currently the only curative therapeutic option.[3] However, few patients have potentially HLA-matched siblings; the incidence of graft failure in the haploidentical setting remains high, and the risk of graft-versus-host-disease is considerable in HLA-matched unrelated HCT.[3] Gene therapy via engineering autologous hematopoietic stem/progenitor cells with corrective approaches has significant potential for a cure, and clinical safety and efficacy trials are ongoing in both diseases.[4] However, significant challenges persist, including preserving the fitness of HSCs for gene modification in patients with severe disease, mobilizing enough HSCs, and maintaining engraftment in a BM niche exposed to recurrent injury. Although the BM is the organ targeted for a cure, little is currently known about how the cumulative effects of hemolysis, iron accumulation, and inflammatory signals caused by these hemoglobinopathies damage the BME and impair HSC functions.

In this elegant study, Aprile and colleagues are the first to test whether β-thalassemia