Harnessing Epigenetic Signatures for Classification of Waldenström macroglobulinemia

Waldenström macroglobulinemia (WM) is a clinically and biologically heterogeneous disease comprised of a complex mixture of lymphocytic and plasmacytic cellular phenotypes. An improved methodology for detecting these states and a deeper understanding of their underlying biology would improve our understanding of the disease. Epigenetic changes occurring during B cell differentiation generate distinct DNA methylation signatures across B cell subsets, including memory B cells (MBCs) and plasma cells (PCs). In previous work, we uncovered that WM patients naturally segregate into two distinct groups according to DNA methylation patterns related to normal MBC and PC profiles with distinct biological features. Here, we expanded our analysis by performing DNA methylation analysis of three independent sample cohorts, totaling 155 WM patients. We validated the existence of the two distinct epigenetic subtypes of WM, identifying 77 MBC-like and 64 PC-like WM patients in the combined cohort, and validated previous differences between the subtypes, including more frequent *CXCR4* mutations and 6q deletions in MBC- and PC-like WM, respectively. Additionally, we have identified novel differences including trisomy 18/+18q, trisomy 3/+3q, and female sex as significantly elevated in MBC-like WM, whereas the frequency of *MYD88*-L265P mutations and +6p was significantly decreased MBC-like WM. To further explore the basis for DNA methylation patterns in WM subtypes, WM patients were analyzed in the context of other B cell malignancies using a DNA methylation-based atlas of >800 B-NHL patients representing 12 distinct entities. WM subtypes are distinct compared to all other entities; however, we surprisingly found that 18% of patients displayed DNA methylation patterns mapping to other B cell malignancies, including mantle cell lymphoma, splenic marginal zone lymphoma, chronic lymphocytic leukemia, and multiple myeloma. *MYD88* wild-type WM patients more often mapped to other B cell malignancies. We have further built a classifier based on DNA methylation profiles to identify WM and its subtypes within the context of other B cell malignancies as the basis for a novel diagnostic tool. Lastly, we have developed a method for WM classification using nanopore sequencing that identifies MBC-like and PC-like epigenetic states, including the ability to detect subclonal heterogeneity of DNA methylation states within samples. Together these findings illustrate a novel approach to subclassify WM patients using patterns of DNA methylation revealing divergent molecular signatures among WM patients, with important implications for diagnosis, classification and understanding of the underlying mechanisms of WM.