Genomic Landscape of Waldenström Macroglobulinemia

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KEYWORDS

• Genomics • Waldenström macroglobulinemia • MYD88 • CXCR4 • Pathogenesis • Treatment

KEY POINTS

- Next-generation sequencing has revealed recurring somatic mutations in Waldenström macroglobulinemia (WM).
- Common mutations include MYD88 (95%–97%), CXCR4 (30%–40%), ARID1A (17%), and CD79B (8%–15%), which are typically found in MYD88-mutated patients.
- The genomic findings provide important insights into the pathogenesis, prognostication, and treatment outcome in WM.

INTRODUCTION

Next-generation sequencing has identified recurring somatic mutations in myeloid differentiation primary response 88 (MYD88), as well as C-X-C chemokine receptor type 4 (CXCR4), AT-rich interactive domain 1A (ARID1A), and cluster of differentiation (CD)79, along with copy number alterations impacting regulatory genes that affect nuclear factor kappa-B (NFKB), Bruton tyrosine kinase (BTK), B-cell lymphoma 2 (BCL2), and apoptosis in chromosome 6q, and elsewhere.[1] Although most patients with Waldenström macroglobulinemia (WM) (95%) carry an MYD88 mutation, those that do not show a more aggressive disease course and many somatic mutations that overlap with those found in diffuse large B-cell lymphoma (DLBCL).[2,3] Herein we discuss the genomic landscape of WM, and the impact of underlying genomics on disease presentation, transcriptional changes, treatment outcome, and overall survival.

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MUTATIONS IN MYD88

A recurring somatic mutation in MYD88 (MYD88 L265P) was identified in 91% of patients with WM by paired tumor/normal whole-genome sequencing, and subsequently confirmed by Sanger sequencing and allele-specific polymerase chain reaction (PCR) assays.[4–9] By sensitive allele-specific PCR testing, MYD88 L265P was expressed in 93% to 97% of patients with WM, including sorted CD19+ CD138− B cells, as well as CD19− CD138+ plasma cells that make up the malignant clone in WM. In addition, non-L265P MYD88 mutations have also been identified in patients with WM, including S219C, M232T, and S243N, although their expression estimates are much lower at 1% to 2%.[8] MYD88 mutations also are detectable in 50% to 80% of immunoglobulin (Ig)M but not IgG or IgA monoclonal gammopathy of undetermined significance (MGUS), suggesting an early oncogenic role for WM pathogenesis.[5–7] Patients with IgM MGUS with mutated MYD88, as well as a higher mutated allele burden for those who are MYD88 mutated, may identify those patients with IgM MGUS at higher risk of progression to WM.[5,10]

The MYD88 L265P mutation also can be detected by allele-specific PCR in peripheral blood samples, particularly in treatment-naïve patients with WM. Prior therapy with B-cell–depleting agents though can greatly decrease the detection of MYD88 L265P in peripheral blood samples.[11] In addition, MYD88 L265P can be found in cerebrospinal fluid and pleural effusions, providing a means of detecting WM disease in patients with central nervous system or pleural disease involvement.[12,13]

Structural events on chromosome 3p can increase the allele burden of mutated MYD88 in 12% to 13% of untreated patients, and upward to 25% of previously treated patients, and segue with CXCR4 mutations in the latter population.[4,8] Deletions of the wild-type (WT) MYD88 allele, and amplifications of the mutant MYD88 allele, have been observed, although acquired uniparental disomy events are the most common reason for homozygous mutated MYD88.[4,8,14] The clinical significance of these structural changes remains to be clarified, but may be relevant to time from diagnosis and ibrutinib response.

The presence or absence of MYD88 mutations discerns 2 distinct populations of patients with WM. Patients lacking MYD88 mutations show histologically similar disease to MYD88-mutated patients but present with more aggressive disease, manifested in decreased overall survival, higher risk of disease transformation, and lack of response to ibrutinib (discussed later in this article).[2,15]

MYD88 is an adaptor protein that interacts with the Toll-like receptor (TLR) and interleukin (IL)-1 receptor families, and undergoes dimerization on receptor activation. The dimerization of MYD88 provides a scaffold for the recruitment of other proteins to a “Myddosome” that triggers downstream signaling leading to NFKB activation (**Fig. 1**).[16] Both IL-1 receptor-associated kinase 1 (IRAK1)/IRAK4 and BTK are components of the “Myddosome” and trigger NFKB independent of IRAK1/IRAK4.[17,18] Recruitment and activation of the IRAK and BTK molecules can be blocked by either knockdown or inhibition of MYD88 that leads to apoptosis of MYD88-mutated WM cells.[17–19] Mutated MYD88 can also upregulate transcription of HCK, an SRC family member that is normally downregulated in late stages of B-cell ontogeny.[20] Mutated MYD88 can also transactivate HCK through production of IL-6. Activated HCK in turn triggers prosurvival signaling of mutated WM cells through BTK, PI3K/AKT, and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)1/2.[20] Both BTK and HCK are potent targets of ibrutinib, which has shown remarkable activity in patients with MYD88-mutated WM.[15,20]

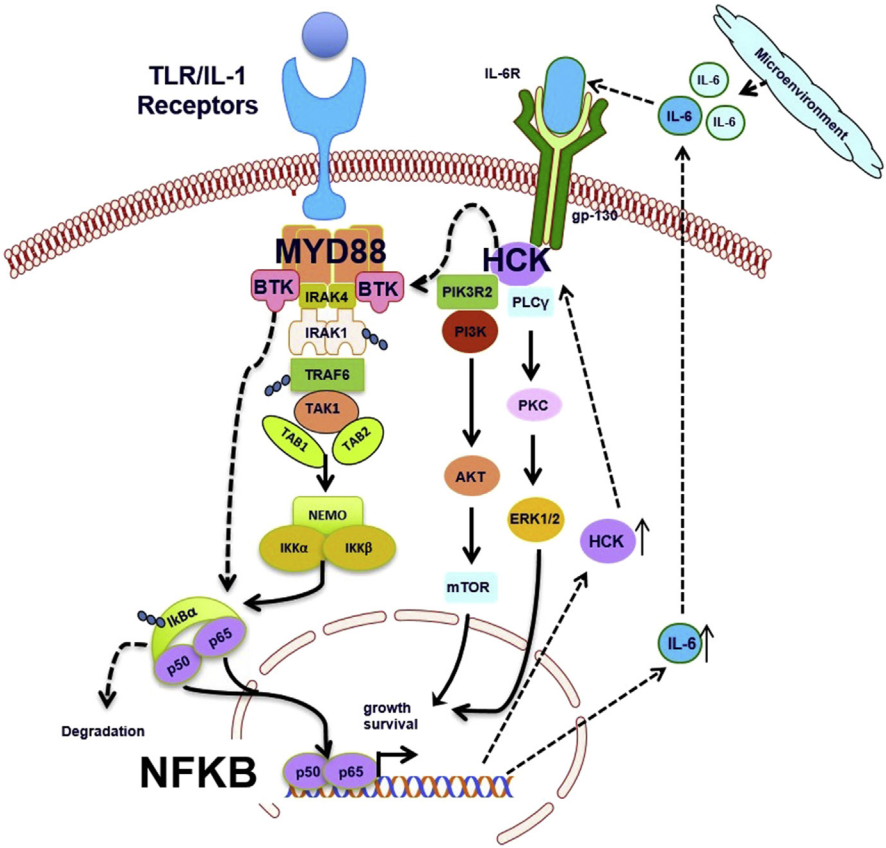

Fig. 1. Mutated MYD88-related signaling in WM. Mutated MYD88 transactivates NFKB through divergent pathways that include IRAK1/IRAK4 and BTK. Mutated MYD88 also triggers transcription and activation of the SRC family member HCK. Activated HCK can then trigger BTK, AKT, and ERK1/2-mediated progrowth and survival signaling in WM cells.

MUTATIONS IN C-X-C CHEMOKINE RECEPTOR TYPE 4

Mutations in the C-terminal domain of CXCR4 are present in up to 40% of patients with WM, and although they almost always occur with MYD88 mutations, some patients with WT MYD88 can also express these mutations.[2,21–24] CXCR4 mutations are essentially unique to WM, as they have not been described so far in other diseases, with the exception of a few marginal zone B-cell lymphoma (MZL) and activated B-cell-like (ABC) DLBCL cases. Germline mutations in the C-terminal domain of CXCR4 are also present in patients with WHIM (autosomal dominant warts, hypogammaglobulinemia, infection, and myelokathexis) syndrome.[25] In patients with WHIM syndrome, activation of CXCR4 by its ligand CXCL12 causes extended chemotactic signaling and neutrophil sequestration in the bone marrow (myelokathexis) and impairment of lymphocyte development.[25] In WM, more than 30 different nonsense and frameshift mutations in the C-terminal domain of CXCR4 have been described. Mutations in the C-terminal domain of CXCR4 lead to loss of regulatory serines, which undergo phosphorylation following CXCR4 receptor activation by CXCL12.[25] With the rest of this g-protein–coupled receptor (GPCR) left intact, mutated CXCR4 remains

fully competent in downstream signaling via g-proteins and beta-arrestins, resulting in the constitutive PI3K/AKT and MAPK/ERK1/2 signaling. Despite the autonomous prosurvival signaling of CXCR4 mutations, inhibition of MYD88 led to apoptosis of both WT and mutated CXCR4-expressing WM cells, suggesting primacy of mutated MYD88 survival signaling in WM.[26]

Unlike MYD88, CXCR4 mutant clonality is highly variable. Multiple CXCR4 mutations also can exist within individual patients, which occur in separate clones or are present as compound heterozygous events.[22] The subclonal nature of CXCR4 mutations relative to MYD88 suggests that these mutations occur after MYD88, although this is likely to be an early event in WM pathogenesis given their detection in patients with IgM MGUS.[22,23] Clonal 6q deletions, which are found in 40% to 50% of patients with WM, appear exclusive of CXCR4.[27]

Like MYD88, the presence of CXCR4 somatic mutations can impact disease presentation in WM. Patients with CXCR4 mutations present with a significantly lower rate of adenopathy, and those with CXCR4 nonsense mutations have increased bone marrow disease, serum IgM levels, and/or symptomatic hyperviscosity. Despite differences in clinical presentation, CXCR4 mutations do not appear to adversely impact overall survival in WM.[2,28]

In vitro modeling of WM cells transduced with mutated CXCR4 showed increased drug resistance in the presence of CXCL12 to multiple therapeutics, including bendamustine, fludarabine, bortezomib, idelalisib, and ibrutinib.[26,29,30] Importantly, these studies showed that resistance mediated by mutated CXCR4 could be reversed by use of CXCR4 blocking agents, and a clinical trial examining the CXCR4 antagonist ulocuplumab along with ibrutinib has been initiated in CXCR4-mutated WM.

OTHER RECURRING MUTATIONS

Somatic mutations in ARID1A are present in 17% of patients with WM, nonsense, and frameshift variants.[1] Patients with ARID1A and MYD88 L265P mutations showed greater bone marrow disease involvement, and lower hemoglobin and platelet count. ARID1A and its frequently deleted homologue ARID1B (discussed later in this article) are on chromosome 6q.[1,27] Both are switch/sucrose nonfermentable (SWI/SNF) family members, and serve as chromatin-remodeling genes, thereby modulating gene regulation. Although still poorly understood, ARID1A can modulate TP53, and is thought to act as an epigenetic tumor suppressor in ovarian cancer.[31–33] CD79A and CD79B can be found in 8% to 12% of patients with WM. Both are components of the B-cell receptor (BCR) pathway, and can form heterodimers with each other.[33,34] The CD79A/B heterodimer associates with the immunoglobulin heavy chain required for cell surface expression of BCR, and BCR induced signaling.[33,34] Activating mutations in the immunotyrosine-based activation motif (ITAM) of CD79A and CD79B have been reported in ABC DLBCL, and trigger spleen tyrosine kinase (SYK), phospholipase Cγ2 (PLCγ2), and BTK. The role of BCR in WM pathogenesis remains to be clarified, although enhanced BCR signaling was observed in WM cells stimulated with BCR activating agents.[35] Deletions of LYN that are found in 70% of patients with WM could contribute to hyperresponsive BCR signaling as informed by lyn−/− transgenic mice.[36] Although mutations in both CD79A and CD79B are mainly found in patients with MYD88 mutation, a CD79B mutation was observed in a patient with MYD88 WT WM.[3,23,37] In one study, CD79A and CD79B were nearly exclusive to CXCR4 mutations, suggesting that 2 distinct MYD88 mutated populations may exist with WM.[23] In a small series of patients with WM, the coexpression of CD79B and MYD88 mutations associated with disease transformation.[38]

MUTATIONS IN MYD88 WILD-TYPE WALDENSTRÖM MACROGLOBULINEMIA

A small number of patients with WM (5%) lack mutations in MYD88, including non-L265P mutations. Their disease course is marked by an increased risk of disease transformation and shorter overall survival.[2] Moreover, these patients show little activity to the BTK inhibitor ibrutinib. These findings point to fundamental differences in underlying genomics. Whole-exome sequencing identified somatic mutations in patients with MYD88 WT WM that are predicted to trigger NFKB (TBL1XR1, PTPN13, MALT1, BCL10, NFKB1, NFKB2, NFKBIB, NFKBIZ, and UDRL1F), impart epigenomic dysregulation (KMT2D, KMT2C, KDM6A), or impair DNA damage repair (TP53, ATM, and TRRAP).[3] Predicted NFKB activating mutations were downstream of BTK, and many overlapped with somatic mutations found in DLBCL.[39,40]

Transcriptome studies revealed a distinctive transcriptional profile in patients with MYD88 WT WM, although most differentially expressed genes overlapped with those found in MYD88-mutated WM.[3] These findings are likely to explain many of the uniform characteristics shared among patients with WM, regardless of their underlying MYD88 mutation status.

COPY NUMBER ALTERATIONS

Copy number alterations are common in patients with MYD88-mutated WM, and involve both chromosome 6q, and non–chromosome 6q regions.[27,41] In chromosome 6q, loss of genes that modulate NFKB activity (TNFAIP3, HIVEP2), BCL2 (BCLAF1), apoptosis (FOXO3), BTK (IBTK), plasma cell differentiation (PRDM1), and ARID1B occur. Non–chromosome 6q genes that are commonly deleted include ETV6, a transcription repressor; BTG1, that often is deleted in DLBCL, and associated with glucocorticoid resistance in acute lymphocytic leukemia; as well as LYN, a kinase that regulates BCR signaling. PRDM2 and TOP1 that participate in TP53-related signaling are also deleted in many patients with WM.[41] In contrast to $MYD88^{MUT}$ WM, recurring copy number alterations are rare in $MYD88^{WT}$ WM, including loss of chromosome 6q.

GENE EXPRESSION PROFILING

Transcriptome studies have allowed gene expression analysis based on underlying somatic gene mutations. Comparison of MYD88-mutated WM and healthy donor (HD) B cells showed increased expression of the VDJ recombination genes DNTT, RAG1, and RAG2, BCL2, as well as CXCR4 pathway genes.[42] The latter support a role for CXCR4 signaling regardless of underlying CXCR4 mutation status. Based on expression and pathway analysis studies, modulation of MYD88 signaling in context of CXCR4 mutations associated with the downregulation of TLR4, and increased transcription of IRAK3, the IRAK4/IRAK1 inhibitor. WM cells derived from patients with MYD88 WT also showed overexpression of DNTT, RAG1, RAG2, CXCL12, and VCAM1, but not BCL2 in distinction to MYD88-mutated disease. The latter may be clinically significant because the BCL2 inhibitor venetoclax is active in WM, although the importance of MYD88 mutation status remains to be clarified and may provide a biological marker for its use in WM.[43] Gene set enrichment analysis also indicated a downregulation of NFKB-induced TNFA signaling in patients with MYD88 WT relative to patients with MYD88-mutated WM, and an increase in AKT/mammalian target of rapamycin (MTOR) signaling. The latter is of clinical interest for exploration of PI3K or MTOR inhibitors in MYD88 WT WM.

SUMMARY

Next-generation sequencing has revealed recurring somatic mutations in WM that include MYD88, as well as CXCR4, ARID1A, and CD79B that typically accompany MYD88-mutated disease. In contrast, the genomic landscape of MYD88 WT WM includes mutations in many genes that are found in DLBCL, and include NFKB activating mutations that are downstream of BTK, as well as mutations in chromatin-modifying genes and DNA damage repair that are found in MYD88 WT disease. The genomic findings provide important insights into the pathogenesis, prognostication, and therapeutic development for WM.

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