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Genetic Factors and Pathogenesis of Waldenström's Macroglobulinemia

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Abstract

Waldenström's Macroglobulinemia (WM) is an indolent but incurable B-cell malignancy. Over the last decade, advances in the molecular field brought about by the use of high-throughput genomic analyses—including array-based comparative genomic hybridization and massively parallel genome sequencing—have considerably improved our understanding of the genetic basis of WM. Its pathogenesis, however, remains fragmented. Important steps have been made in elucidating the underlying aberrations and deregulated mechanisms of the disease, and thereby providing invaluable information for identifying biomarkers for disease diagnosis, risk stratification and therapeutic approaches. Herein, we review the genetic basis of the disease.

Keywords

Waldenström's macroglobulinemia; array-based comparative genomic hybridization; massively parallel DNA sequencing; MYD88; genetics

Introduction

Waldenström's Macroglobulinemia (WM) is an indolent B-cell malignancy characterized by a lymphoplasmacytic infiltration of the bone marrow and monoclonal immunoglobulin M (IgM) protein hypersecretion. [1] The overall age-adjusted incidence of WM is 0.38 cases per 100,000 persons per year, increasing with age to 2.85 in patients above 80 years. There is a male predominance throughout all age groups and the disease has a higher incidence in

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Compliance with Ethics Guidelines

Conflict of Interest

Jorge Monge Urrea, Esteban Braggio and Stephen M Ansell declare that they have no conflict of interest

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whites than non-whites. [2] There is an increased incidence of second cancers in patients with WM, both solid tumors and hematologic malignancies. [3,4] WM is classified as an immunosecretory disorder with an underlying lymphoplasmacytic lymphoma according to the World Health Organization (WHO) classification. [5] The lymphoplasmacytic infiltration consists of hypermutated post-germinal center B-cells ranging from small B-lymphocytes to fully differentiated plasma cells. The lymphocytic component typically has high levels of surface CD19, CD20 and immunoglobulin light chain expression, but lacks CD10 expression. [6] In some cases, lymphocytes may express CD5, although not as strongly as in chronic lymphocytic leukemia (CLL) or mantle cell lymphoma. [6] The plasmacytic component expresses immunoglobulin light chains, and is CD138 positive while expressing lower levels of CD19, CD20, and PAX5 than the lymphocytic component. [6] IgM levels correlate in part with the degree of plasma cell differentiation. [7]

Patients with WM may present in a variety of ways. About 30% are asymptomatic (smoldering WM). [8] The clinical features of symptomatic WM patients include those due to bone marrow infiltration (anemia, pancytopenia), extramedullary infiltration (hepatomegaly, splenomegaly, lymphadenopathy), hyperviscosity (oronasal bleeding, retinal hemorrhage with blurring or loss of vision, headache, vertigo, ataxia) and IgM monoclonal proteins (peripheral neuropathy). [9] The most common presenting symptom is fatigue due to anemia (mean hemoglobin value at diagnosis 10 g/dL). [10]

The diagnosis of WM involves evaluating the clinical features, and performing a serum protein analysis and a bone marrow biopsy. A diagnosis can be made with (1) an IgM monoclonal gammopathy in the serum (irrespective of IgM concentration), (2) 10% lymphoplasmacytic infiltration of the bone marrow, and (3) supportive immunophenotypic studies. Asymptomatic patients that fulfill these criteria are classified as having smoldering WM. [11,12] Asymptomatic patients with only an IgM monoclonal protein and less than 10% bone marrow infiltration can be classified as having IgM secreting monoclonal gammopathy of undetermined significance (IgM-MGUS). [13]

Several prognostic models have been developed: Kyle and colleagues described the risk of subsequent progression in asymptomatic WM [14]; the International Prognostic Scoring System for WM (IPSSWM) is used for risk stratification at the time of first-line therapy [15]; Gobbi et al. demonstrated the importance of at least one criterion for initiating therapy in WM [16]; while Treon and colleagues determined the prognostic role of the degree of response on progression-free survival [17]. Characteristics that adversely affect prognosis in WM include age (>65 years), increased beta-2 microglobulin, organomegaly, anemia, thrombocytopenia, low albumin, high serum monoclonal protein concentration, and high serum free light chain concentration. [18–20] Increased levels of von Willebrand factor have been identified as an independent adverse prognostic factor, as well as with a microenvironment that stimulates growth and survival of tumor cells via increased vascular endothelial growth factor (VEGF) and bone marrow microvessel density. [21,22]

The goal of therapy in WM is the relief of symptoms and to minimize the risk of organ damage. Indications for therapy include symptomatic hyperviscosity, neuropathy, symptomatic adenopathy or organomegaly, amyloidosis, cryoglobulinemia, or cytopenia. [23] Symptoms due to hyperviscosity should be managed with plasmapheresis before starting primary therapy. [23] The decision to treat patients with WM is based on age, presence of comorbidities, symptomatology and progression of the disease. First-line therapy includes anti-CD20 monoclonal antibodies, either as a single agent or in combination with alkylating agents or nucleoside-analogues. [23] The median 5-year survival for low, intermediate and high risk patients is 92%, 63% and 27%, respectively.

[15, 24] In certain high-risk patients, high-dose chemotherapy followed by autologous or allogeneic hematopoietic cell transplantation may increase progression-free survival. [25]

Patients with IgM-MGUS are at an increased risk of 1.5% per year of progressing to WM, while the rate is 12% per year (for the first five years) for patients with smoldering WM. [26,27] While the majority of cases of WM are sporadic, there are several studies showing a familial linkage in high-risk families predisposing to the disease, finding the strongest evidence of linkage in chromosomes 1q, 3q, 4q and 6q, when combining IgM-MGUS and WM. [28–32] Carriers of the hyperphosphorylated stage of paratarg-7 (pP-7), a protein of unknown function, have been associated with a 6-fold increase in the development of IgM-MGUS/WM. Because pP-7 is inherited as a dominant trait, these findings might help identify family members at increased risk. [33]

Genetic analysis has been increasingly used to understand the pathogenesis of WM. In this manuscript we provide an overview of the genetic landscape of WM.

Practical Aspects of Genetic Testing

The genetic analysis of WM employs comprehensive, high-resolution tests, at the DNA (aCGH/SNP arrays, DNA sequencing), RNA (gene expression profiling, mRNA sequencing) and microRNA (microRNA expression array) level. It is critical that molecular tests be performed in purified tumor cells, in order to avoid false negative results as a consequence of the contamination with non-tumor cells.

The immunophenotype of WM consists of pan-B-cell markers (CD19, CD20, CD22), cytoplasmic immunoglobulin (cIg), FMC7, CD38, and CD79a. [11,34,35] Plasma cells in patients with WM differ from normal and multiple myeloma (MM) cells because, although they are CD38+, they are commonly CD19+, CD20+, CD45+, and CD56-. [35] If a purified clonal population is required, multiparametric sorting should be considered, keeping in mind that the biggest limitations of this method are its complexity and the low cell count obtained for further molecular studies. Normal B-cells (CD19+) and plasma cells (CD138+) may be used as controls and should be serially enriched by using anti-CD19 and, subsequently, anti-CD138 beads. Even though most molecular techniques may tolerate moderate levels of contamination with non-tumor cells (20–30%), exceeding this threshold will yield compromised results. Either sorting or immunofluorescent detection of cytoplasmic immunoglobulin M is needed to identify tumor cells during fluorescence in situ hybridization (FISH) analysis (cIgM-FISH). [36]

High-throughput approaches usually need a minimum of 1–3 ug of DNA/RNA, varying according to the technique used. This excludes a significant number of patients due to insufficient DNA/RNA obtained from the enriched fraction. Quality requirements are another restrictive factor, especially for RNA assays, for which array-based and next-generation sequencing techniques require an RNA integrity higher than 7.5. For DNA assays, a 260/280 ratio of 1.75–1.85 and a 260/230 ratio >1.9 is recommended.

Initial genomic characterization using conventional cytogenetics and FISH

As a hypoproliferative disease, the genetic study of WM is hampered by its low rate of replication. This has been addressed by the use of techniques focusing on the study of interphase nuclei. Conventional cytogenetics (CC) provided the first insights into whole genome analysis of WM [37,38], however, the rate of successful analysis was low (<30% of cases) due to the low resolution of the technique and the need for tumor cell division. The absence of tumor cell division has been overcome by the development of FISH, although this only provides a targeted validation of previously recognized abnormalities. Initial CC

studies reported deletion of 6q to be the most common recurrent chromosomal abnormality, seen in nearly 50% of patients. [37] Further studies, by FISH analysis, suggested a minimal deleted region (MDR) at 6q23–24.3. [39] One of the largest cohorts of patients with WM (N=172) to date showed abnormalities in 47% by CC and FISH. [40] Of note, 35% of cases with an abnormal karyotype showed chromosomal translocations. [40] After combining both CC and FISH, deletion of 6q was observed in 30%, trisomy 18 in 15%, deletion of 13q14 in 13%, trisomy 4 in 8%, deletion of 17p13 (*TP53*) in 8%, deletion of 11q22 (*ATM*) in 7%, trisomy 12 in 4% and 14q32 (IgH) translocations in 2%. [40]

Deletion of 6q cannot be used to differentiate WM from other B-cell malignancies, since it is present in several of them, such as diffuse large B-cell lymphoma (DLBCL), marginal zone B-cell lymphoma (MZL), MM and CLL. [41–43] IgH translocations, however, are rare in WM, occurring in less than 3% of cases. [39,40,44,45] The other recurrent chromosomal abnormalities, like trisomies 3, 12 and 18, and deletion of 7q, are mostly shared between WM and other low-grade B-cell malignancies, such as CLL, MZL and mucosa-associated lymphoid tissue (MALT) lymphomas. [41,46,47] On the other hand, trisomy 4 seems to be a unique finding in WM, where it may be the only genetic abnormality found by CC or FISH. [41,45,46,48] Added efforts using high-resolution techniques have not succeeded in finding a minimal gained region of chromosome 4. The deletion of 6q has been associated with clinical features and patient outcome, albeit that this finding remains controversial. Deletion of 6q was associated with an albumin <3.5 g/dL ($p<0.0001$) and a $2M >3\text{mg/L}$ ($p=0.04$), but was not associated with response rate, progression-free survival, disease-free survival or overall survival. [40] In a different study, patients with deletion of 6q by FISH were associated with higher $2M$ levels ($p=0.001$), anemia ($p=0.01$) and lower albumin ($p=0.001$). [49] Two other studies reported that WM patients with deletion of 6q had lower IgM production; but a significant correlation between deletion 6q, disease progression or prognosis was not found. [50,51] The implications of trisomy 4 have not been elucidated, but a study has suggested that 4q may be a factor in increased susceptibility to WM after conducting a genome-wide analysis in 11 high-risk families with a total of 122 individuals. [29] A high non-parametric linkage was found on cytoband 4q33–q34, suggesting both linkage and common susceptibility between IgM-MGUS and WM patients. [29] This sets the stage for further studies aimed at clarifying the role of chromosome 4 in the etiology of WM.

Array-based studies

Array-based studies have enhanced our knowledge of chromosomal abnormalities in WM without the need for tumor cell division. Using array-based comparative genomic hybridization (aCGH) 83% of WM patients (35 out of 42 cases) were found to have chromosomal abnormalities, with a median of 3 abnormalities per patient and 16 abnormalities found in over 5%. [46] Partial or whole deletion of 6q has been the most frequently observed chromosomal abnormality in WM (Table 1), while four separate MDR in 6q have been seen across numerous B-cell malignancies. Two of these MDR in WM include *PRDM1* (BLIMP) and *TNFAIP3*. [46] *PRDM1* is known to repress cell proliferation and down regulate *PAX5*, which in turn suppresses XBP1. Although *PAX5* has not been found to be mutated or over-expressed in WM, XBP1 has been proven to be over-expressed in 61% of WM patients. [52] Inactivation of the tumor suppressor gene *TNFAIP3* leads to the constitutive activation of the NF- κ B signaling pathway. [46,53,54] While biallelic inactivation of *TNFAIP3* was found in approximately 5% of patients with WM, a monoallelic deletion was found in nearly 40%, resulting in lower transcript expression than in cases where both gene copies were detected. Using aCGH, 16.6% of patients with WM were found to have a gain of 6p, always secondary to a deletion of 6q, making it the second most frequent abnormality. [46]

Inactivation of *TRAF3* (14q32.32), though rare in WM (~5%), has noteworthy implications by resulting in the constitutive activation of NF- κ B signaling pathway. [46] Other B-cell malignancies, including MM and DLBCL, with this abnormality also demonstrate increased activation in the NF- κ B signaling pathway. [53,55,56]

Other recurrent deletions, affecting cytobands 13q14 and 17p13, are mostly seen in advanced WM with an approximate 10% prevalence by aCGH (Table 1). The MDR in 13q14 differs from that in MM but is similar to the one found in CLL and MZL, comprising the microRNAs *MIRN15A* and *MIRN16-1*. [43,46,48,57] Both miRNAs have a tumor suppressor effect by negatively regulating *BCL2*. [58] *TP53*, included in 17p13, acts as a gatekeeper, and when lost, p21 is not activated and is unable to interact with CDK2 to arrest the cell cycle. [59] All of these chromosomal abnormalities play a potential role in the pathogenesis of WM.

Massively Parallel Sequencing

The myeloid differentiation primary response gene 88 (*MYD88*) is an adaptor protein involved in Toll-like receptor (TLR) and NF- κ B pathways. [60–62] Oncogenically active *MYD88* mutations were first described in nodal DLBCL, present in 29% of the activated B-cell-like subtype, but rare in the germinal center B-cell-like subtype. [63]

Whole-genome sequencing performed in 30 WM patients showed a *MYD88* mutation leading to a leucine to proline substitution in codon 265 (L265P). [64] This study showed the presence of *MYD88* L265P in 91% of patients with WM; in contrast, *MYD88* L265P was present in only 10% of IgM-MGUS, 7% of patients with MZL and absent in myeloma, including IgM-secreting myeloma. Recently, an allele-specific polymerase chain reaction (AS-PCR) assay was developed to obtain a cost-effective and efficient detection of *MYD88* L265P. By using AS-PCR, the *MYD88* L265P was identified in 100% of patients with WM (N=58), compared with 47% of IgM-MGUS (36/77 patients), and 6% (5/84) of splenic MZL. [65] The lower prevalence seen in IgM-MGUS of *MYD88* mutation suggests either an association with disease progression or different subtypes of IgM-MGUS, with only some progressing to WM. On the other hand, the sequencing data suggest the use of *MYD88* L265P as a potential biomarker to differentiate WM from other B-cell malignancies. [64] The presence of *MYD88* L265P was associated with higher BM involvement ($p=0.01$) and serum IgM levels ($p=0.05$). [66] Furthermore, it was found that patients with IgM-MGUS carrying *MYD88* L265P had a higher risk of disease progression (OR 4.7, 95% CI: 0.8–48.7, $p=0.04$). [65] These studies have set the groundwork for *MYD88* L265P analysis to become a reliable tool for diagnosis, prognosis and response assessment in WM.

The *MYD88* L265P mutation promotes cell survival by spontaneously assembling a protein complex containing IRAK1 and IRAK4, leading to IRAK4 kinase activity, phosphorylation of IRAK1, NF- κ B pathway activation, and secretion of IL-6, IL-10 and interferon- γ (Figure 1). [63] The development of IRAK4 kinase inhibitors and other upstream proteins in this pathway may provide a novel therapeutic opportunity in the treatment of WM and other B-cell malignancies. [63] [64]

Massive parallel sequencing has identified additional somatic mutations in 10 to 23% of WM cases in seven other genes (*CXCR4*, *TAP2*, *LRP1B*, *MXLN*, *ARID1A*, *HIST1H1E* and *RAPGEF3*) but further studies are needed to validate these findings. [64]

Gene expression profiling analyses

Two comprehensive gene expression analyses provided the basis of the WM transcriptional signature and its similarities and differences with CLL, MM, normal B-cells, and normal

plasma cells [67,68]. These studies specifically highlight similarities between malignant WM cells and CLL compared with MM cells [67]. Furthermore, the gene expression profiling of the B-cell and plasma cell populations of WM is different from their CLL and MM counterparts. [67,68].

Differences between WM and other B-cell neoplasias, such as CLL and MM, can be extrapolated and perhaps used as disease markers. Thus, CD1c, a transmembrane glycoprotein that is structurally related to the major histocompatibility complex (MHC) proteins, was significantly higher in WM than in MM or CLL, and could be a potential marker of WM [67]. Probably the most remarkable hit distinctive of WM found in expression studies is the high expression level of interleukin 6 (IL-6) compared to related B-cell malignancies and normal B-cells [67,68]. Increased IL-6 expression in WM is associated with activation of JAK/STAT and MAPK pathways and stimulates IgM secretion [67,69]. Additional studies are needed to better understand the pathogenic role IL-6 and JAK/STAT pathways play in WM.

MicroRNAs

In WM, miRNA-155, -206 and -9, among others, are of particular interest. [70] A comparison of miRNA profile between BM CD19+ cells from WM patients and CD19+ cells isolated from BM and peripheral blood of healthy donors, helped to identify a WM-specific miRNA signature comprised of increased expression of miRNA-155, -184, -206, -363, -494 and -542-3p, and decreased expression of miRNA-9 (ANOVA, $p < 0.01$). [71] The increased expression of those miRNAs was significantly elevated in patients with a poor outcome predicted by the IPSSWM. [71] After treatment with rituximab, perifosine and bortezomib, WM cell lines showed a reduction in miRNA-155, -184, -206, -363, -494 and -542-3p, and increased expression of miRNA-9. [71] The changes in expression level affect downstream targets of these miRNAs, including the RAS oncogene family, transcription factors, cell-cycle and anti-apoptotic regulators. [71] miRNA-155 has been shown to regulate proliferation and growth of WM cells in vitro and in vivo, through the inhibition of MAPK/ERK, PI3/AKT, and NF- κ B pathways. [71] Using gene expression profiling, potential target genes for miRNA-155 have been identified, including genes involved in cell-cycle progression, adhesion and migration. [71] miRNA-155 knockdown cells have been demonstrated to have a decrease in Mdm2, resulting in an increase in p53 and CDK inhibitors. [72] The decreased response to stromal derived factor-1 and inhibition of fibronectin adhesion suggest that miRNA-155 has a role in WM cell survival. [72] The use of locked nucleic acid (LNA) anti-miRNA-155 decreased levels of miRNA-155, inhibiting growth of WM cells in vivo and in vitro, providing the rationale for miRNA targeted therapies. [73]

Hypoacetylation is associated with condensed chromatin, leading to the decreased gene transcription, while acetylation is associated with a more open chromatin structure and increased transcription, this balance is maintained by the regulation of the level of histone deacetylase (HDAC) and histone acetyl transferases (HAT). [74] In WM, increased HDAC and decreased HAT expression has been correlated with higher miRNA-206 and lower miRNA-9 levels. [74] The epigenetic modification of increased HDAC activity may impact cell-cycle regulators, including p21 and p53, leading to cell proliferation. [74] These studies highlight the role of miRNAs in the initiation and progression of WM, as well as new therapeutic targets.

Conclusions

From the use of CC and FISH to the development of high-throughput whole-genome techniques, our ability to study the genetic basis of WM has dramatically improved. The most remarkable finding has been the identification of the *MYD88* L265P mutation as a universal event of the disease. This finding not only can be potentially used as a biomarker to differentiate WM from related B-cell malignancies but also identifies potential targets for developing more focused therapeutic approaches. As the study of WM continues, the individual genetic profile of patients may prove to be of utmost importance in determining the most effective therapeutic approaches for patients.

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Figure 1.

MyD-88 dependent TLR signaling pathway: MyD-88 (*) associates with the cytoplasmic domain of TLR, recruiting IRAK1 and IRAK4 to activate TRAF6. TRAF6 then activates the IKK complex (IKK α , IKK β and IKK γ), which phosphorylates I κ B resulting in nuclear translocation of NF- κ B (p50 and p65), which induces expression of inflammatory cytokines.

Table 1

Prevalence and regular function of the most recurrent genetic alterations in Waldenström's Macroglobulinemia patients.

Abnormality	Prevalence (%)	Genes Involved	Regular Function
<i>MYD88</i>	87–100	<i>MYD88</i>	Innate and adaptive immune response
–6q21	38–50	<i>PRDM1</i>	Suppression of cell proliferation
–6q23	38–50	<i>TNFAIP3</i>	Tumor suppressor gene. Negatively regulates NF- B pathway
+4	12–20	Unknown	
+6p	17	Unknown	
+18	17	<i>MALT1, BCL2</i>	Blocks the apoptotic pathway
+3	10	Unknown	
–13q14.3	10	<i>MIRN15A, MIRN16-1</i>	Negatively regulates <i>BCL2</i>
–17p13.1	7–10	<i>TP53</i>	Starts the repair of damaged DNA
–14q32	6	<i>TRAF3</i>	Negatively regulates NF- B pathway