Candidate genes of Waldenström's macroglobulinemia: current evidence and research

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REVIEW

Candidate genes of Waldenström's macroglobulinemia: current evidence and research

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Abstract: Waldenström's macroglobulinemia (WM) is a relatively uncommon, indolent malignancy of immunoglobulin M-producing B cells. The World Health Organization classifies it as a lymphoplasmacytic lymphoma and patients typically present with anemia, hepatosplenomegaly and diffuse lymphadenopathies. Historically, the genetic characterization of the disease has been hampered by the relatively low proliferative rate of WM cells, thus making karyotyping challenging. The use of novel technologies such as fluorescence in situ hybridization, gene array, and whole genome sequencing has contributed greatly to establishing candidate genes in the pathophysiology of WM and to identifying potential treatment targets, such as L265P MYD88. The discovery of microRNAs and the recognition of epigenetics as a major modulatory mechanism of oncogene expression and/or oncosuppressor silencing have aided in further understanding the pathogenesis of WM. Once thought to closely resemble multiple myeloma, a cancer of terminally differentiated, immunoglobulin-secreting plasma cells, WM appears to genetically cluster with other indolent B-cell lymphomas such as chronic lymphocytic leukemia/small cell lymphoma. The relative high incidence of familial cases of WM and other B-cell malignancies has been helpful in identifying high-risk gene candidates. In this review, we focus on the established genes involved in the pathogenesis of WM, with special emphasis on the key role of derangement of the nuclear factor kappa B signaling pathway and epigenetic mechanisms.

Keywords: genetics, familial cases, NF-κB, whole genome sequencing, MYD88

Introduction

Waldenström's macroglobulinemia (WM) is a non-Hodgkin's lymphoma (NHL) typically characterized by bone marrow infiltration by lymphoplasmacytic cells and production of a serum monoclonal immunoglobulin (Ig) M.[1] WM derives its name from Jan Gosta Waldenström, a Swedish physician and luminary who first reported on two patients presenting with oral mucosa bleeding, epistaxis, and diffuse lymphadenopathies.[2] Laboratory evaluation of these patients revealed normochromic anemia, thrombocytopenia, elevated erythrocyte sedimentation rate, and decreased fibrinogen and albumin. Waldenström noticed two crucial differences between this condition and multiple myeloma (MM): first, his patients did not have any bone pain or bone lesions, and, second, the bone marrow was infiltrated by lymphocytic rather than plasmacytic cells.[2,3] Waldenström's preliminary observations have largely been included in the current diagnostic criteria for WM.

WM is defined by the presence of a monoclonal IgM (M spike) of any degree, more than 10% bone marrow involvement by a lymphoplasmacytic infiltrate, and presence of end organ damage directly attributable to either the B-cell clone or the M protein.[3,4]

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The latter criterion distinguishes WM from smoldering WM, in which there is no end organ dysfunction directly attributable to the cancer. IgM monoclonal gammopathy of unclear significance (MGUS), a benign condition with premalignant potential, has been recently reported to precede WM with a rate of progression of 2.0% to 2.5% per year. Three diagnostic criteria are required to be fulfilled for a diagnosis of IgM-MGUS: <10% lymphoplasmacytic infiltration of the bone marrow, <3 g/dL IgM spike, and absence of symptoms or end organ damage attributable to WM.[4]

Ontologically, WM is thought to derive from post-germinal-center B cells that have undergone the process of somatic hypermutation but not isotype class switching.[5] WM cells are characterized by a specific immunophenotype (positive: surface IgM, cluster of differentiation (CD) 19, CD20, CD22, CD25, CD27, FMC7; negative: CD10, CD23, CD103, CD138; and variable CD5 expression), which is helpful in distinguishing WM from other NHL and MM that can appear morphologically similar.

WM is a rare cancer, affecting about six persons per million people, per year in the USA, with incidence increasing with age. Median age at diagnosis is between 63 and 68 years.[6] Men are twice as commonly affected than women and incidence is 2–3 times higher in whites than in blacks.[7]

Patients with WM typically present with nonspecific symptoms such as asthenia and malaise, often related to anemia, which, together with a circulating monoclonal protein IgM detectable on serum protein electrophoresis and/or immunofixation, represents the most common laboratory abnormality.[8] The etiology of the most common presenting symptoms and signs of WM can be explained by several mechanisms: cytopenias secondary to bone marrow infiltration by WM cells and impairment of normal hematopoiesis; hepatosplenomegaly and lymphadenopathy as a result of parenchymal infiltration by malignant cells; and peripheral neuropathy and hyperviscosity related to the nature and quantity of the M protein, respectively. Rarely, WM patients develop amyloid light-chain (AL) amyloidosis from deposition of monoclonal light chain, more often lambda.[9] The latter diagnosis is associated with a dismal prognosis, especially when cardiac involvement is present.

Several negative prognostic factors have been identified in newly diagnosed WM patients and an internationally recognized prognostic algorithm (the International Prognostic Scoring System [IPSS]) was recently developed to help risk-stratify WM patients.[10] Age over 65 years, hemoglobin < 11.5 g/dL, thrombocytopenia < 100,000/μL, β2-microglobulin > 3 mg/L, and monoclonal IgM > 7 g/dL are the adverse prognostic factors on which the IPSS is based.[11]

Although WM is incurable, it is typically characterized by an indolent clinical course. For this reason, active treatment is not recommended until symptoms arise and/or end organ damage ensues. In particular, therapy should commence in the setting of hyperviscosity syndrome and AL amyloidosis. Although the median survival of WM patients has been estimated at around 5 years, this number is likely to overestimate the aggressiveness of the disease for two reasons.[11] First, the estimation includes all causes of mortality rather than cancer-related mortality in a population that is demographically at risk for other morbidities. Second, this figure was obtained by calculating overall survival from the time of commencement of treatment, rather than diagnosis, not accounting thus for the months or years during which WM patients remain under close surveillance without therapy. Indeed, a recently published study found that disease-specific survival in patients with WM exceeds 10 years.[10]

The treatment armamentarium for WM currently includes the anti-CD20 monoclonal antibody rituximab, alkylating agents, purine nucleoside analogs, and the proteasome inhibitor bortezomib, either alone or in combination.[12,13] Bench and clinical research has focused on developing drugs against novel targets important to sustain the maladaptive interaction between the cancer clone and microenvironment, such as the mammalian target of rapamycin (mTOR) or phosphatidil insositol 3 kinase-protein kinase B (PI3K-AKT) pathway and the ubiquitin–proteasome axis.[14,15] Autologous stem cell transplantation is used in selected patients, while allogeneic stem cell transplant is generally not implemented due to high procedure-related morbidity and mortality and the overall indolent course of the disease.[16]

Historically, cytogenetics has been difficult to perform in WM cells due to their low proliferation rate, hampering the possibility of obtaining metaphases. However, the introduction of fluorescence in situ hybridization (FISH), gene array, and whole genome sequencing has provided significant help in the pursuit of a genetic signature for WM that would help understand the pathogenesis of the disease and design target treatment.[17]

In this paper, we review the available data regarding target genes in WM, with a special emphasis on mutations in the nuclear factor kappa B (NF-κB) pathway, especially MYD88, and on recent findings in the field of microRNAs (miRs) and epigenetics. The most common genetic mutations in WM are summarized in Table 1.

Table 1 Outline of the most frequently occurring genetic aberrancies in Waldenström macroglobulinemia (WM) with an emphasis on targeted genes

Chromosomal abnormality	Encoded protein	Incidence	Function	Comments
SNM 3p22.2	MYD88	90%	Activator of canonical NF-κB	• Non-conservative, missense, gain of function, mutation in codon 265 (L265) • Identified via whole genome sequencing
+3	Unknown, possibly BCL6	10%	Unknown	• Shared with MZL, CLL, and MM
+4	Unknown	10%–20%	Unknown	• Familial studies suggest candidate gene to map on 4q33–4q34 • Specific for WM • Occasionally the only genetic mutation
+6p	Unknown	17%	Unknown	• Occurs concomitantly with –6q
6q–		40%–50%		• Del6q discriminates WM from IgM-MGUS
Locus 21	Blimp1		Transcription repressor	• Occurs commonly in MM and NHL
Locus 23	TNFAIP3		Ubiquitin-editing enzyme	
7q–	Unknown	7%		• Shared with MZL, CLL, and MM
11q–	Possibly ATM	10%	Serine-threonine kinase. Activates P53, leading to DNA repair, cell cycle arrest, and apoptosis	• Shared with MZL, CLL, and MM
13q14–	miR 15A and 16-1	10%–13%	Target proteins involved in cell cycle, proliferation and anti-apoptosis	• Shared with MZL, CLL, and MM
14q32–	TRAF3	5%	Inhibitor of noncanonical NF-κB	
17p13–	P53	8%	DNA repairer, cell cycle arrester, and apoptosis generator	• Associated with shortened DFS but not OS
+18	BCL2, Malt1	15%	Anti-apoptotic, NF-κB activator	• Shared with MZL, CLL, and MM • Often associated with +4

Abbreviations: ATM, ataxia telangiectasia mutated; CLL, chronic lymphocytic leukemia; DFS, disease-free survival; Ig, immunoglobulin; MGUS, monoclonal gammopathy of unknown significance; MM, multiple myeloma; MZL, marginal zone lymphoma; NHL, non-Hodgkin's lymphoma; OS, overall survival; SNM, single-nucleotide mutation.

Familial WM cases: an insight into high-risk gene candidates

Although the majority of WM cases are sporadic, familial clustering of WM or co-aggregation with other hematologic malignancies, particularly chronic lymphocytic leukemia (CLL), Hodgkin's lymphoma and NHL, has been reported in as many as 20% of cases.[18,19] Common genetic variants in 152 genes involved in cell proliferation, apoptosis, inflammation, and DNA repair were investigated in families with two or more individuals affected by the same type of lymphoma, resulting in over 1500 genotyped single-nucleotide pairs (SNPs).[20] SNPs in the genes codifying for *B-cell lymphoma 2 (BCL2)*, *interleukin 10 (IL10)*, *tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)*, and *tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAILR1)* were associated with both WM and CLL; SNPs in IL-6 were common between WM and Hodgkin's lymphoma, thus providing a potential explanation for their shared familial clustering.

Family studies also revealed that first-degree relatives of WM patients carry an increased incidence of the premalignant condition IgM-MGUS.[21,22] Linkage studies in such families identified chromosome 1q, 3q, and 4q as potentially responsible for this genetic predisposition. Paratarg-7 (P-7), a ubiquitous protein whose function is unclear, was reported to be the antigenic target of circa 15% monoclonal IgA and IgG in patients with MGUS or MM.[23,24] Similarly, a German group showed the monoclonal protein of patients with WM or IgM-MGUS reacts against a hyperphosphorylated form of P-7 (pP-7) in around 11% of cases.[25] Family linkage analysis showed pP-7 to be inherited according to an autosomal dominant pattern.[26] Given the low prevalence of pP-7 expression in the healthy population (2%), individuals expressing hyperphosphorylated P-7 proved six times more likely to develop WM or IgM-MGUS than the general population, thus suggesting a causative role for this antigenic target.[25]

Similarities and differences between WM and other hematologic malignancies: from karyotype to gene-expression profiling (GEP)

WM shares with several B-cell derived malignancies (marginal zone lymphoma (MZL), CLL, and MM) several

genetic aberrancies such as trisomy 3, trisomy 18, deletion 7q, deletion 11q, and deletion 13q.[27] In contrast to MM and MZL, WM cells only rarely carry a translocation involving the heavy immunoglobulin (IgH) locus (3%).[28] Trisomy 4 is identified in about 10%–20% of WM patients and appears to be specific for WM and occasionally the only genetic aberration noticed.[29] Although no discrete candidate gene has yet been identified on chromosome 4, a genome-wide linkage analysis in individuals from a family with high prevalence of WM showed high nonparametric linkage on 4q33–4q34, suggesting a pathogenic role for the gene(s) contained in this region.[30] Trisomy 18, a frequently found abnormality in MZL, associates with trisomy 4 and is present in about 15% of WM patients.[31] Deletion 17p is typically associated with loss of TP53 and underscores a poor prognosis in CLL, MM, and MZL. Del17p occurs in about 8% of WM patients and correlates with shortened disease-free survival but not overall survival, thus suggesting no clear role for it as a negative prognostic factor in WM.[31]

Contradicting what was previously predicated, GEP in B-cell-derived malignancies showed WM to cluster more closely to CLL than MM.[32,33] Using a class prediction model, four genes were able to discriminate between CLL- and WM-derived cells with 100% accuracy: *lymphoid enhancer-binding factor 1 (LEF1)*, *ataxin 1 (ATXN1)*, *fibromodulin (FMOD)*, and *myristoylated alanine-rich protein kinase C substrate (MARCKS)*. The first three were upregulated in CLL while *MARCKS* was upregulated in WM. GEP also revealed a higher expression of *IL-6* in WM than in both MM and CLL. While the role of IL-6 in mediating cell proliferation and drug resistance in MM has been established, its function in the pathogenesis of WM remains unclear.[34–37] It is widely accepted that IL-6 activates the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway and the transcription factor signal transducer and activator of transcription (STAT) 3 via the tyrosine kinase receptor signaling of Janus kinase 1 and 2. In the absence of any identified activating mutation of the MAPK/ERK pathway in WM, IL-6 is believed to be directly responsible for the chronic activation of this signaling cascade.[38] Moreover, the activation of the Janus kinase/STAT pathway by cytokines such as IL-6 and IL-21 has been recently advocated as the mechanism underlying the hypersecretion of IgM by WM cells, which is a major contributor to WM-related morbidity.[39,40]

Deletion of the long arm of chromosome 6: the most common and first identified karyotype abnormality in WM

The indolent nature of WM is reflected in the low proliferation rate of WM cells, which has historically compromised the success of karyotype analysis with conventional cytogenetics.[41] The in vitro use of immunostimulatory factors and cytokines has been instrumental in increasing the mitotic rate of WM cells and thus the success rate of conventional cytogenetics, which is currently exceeding 80% in several series.[31] In a recently published randomized clinical trial conducted in 174 newly diagnosed WM patients, an abnormal karyotype was identified by conventional cytogenetics in 47% of patients, with translocations being present in about 35% of cases.[31]

Deletion 6q (-6q or del6q) is the first and most frequently identified chromosomal abnormality in WM.[42] Recent studies based on high-resolution array-based comparative genomics hybridization and FISH reported the presence of del6q in about 40%–50% of WM patients.[27,43] Importantly, the presence of this mutation proved to confer an unfavorable prognosis in active WM.[41,44] While frequent in WM, del6q is not exclusive to this disease. Loss of four distinct minimal deleted regions on the long arm of chromosome 6 was detected in several B-cell-derived malignancies, including MM and MZL.[45]

More sophisticated techniques such as array-based comparative genomics hybridization have identified *B lymphocyte-induced maturation protein 1* (*BLIMP1*) and *tumor necrosis factor α-induced protein 3* (*TNFAIP3* or *A20*) as candidate tumor suppressor genes mapping in two distinct minimal deleted regions on the long arm of chromosome 6.[32,46] The function of *BLIMP1* and *TNFAIP3* is discussed following.

BLIMP1: an oncosuppressor crucial for plasma cell differentiation

BLIMP1, also known as positive-regulatory domain I binding factor 1 or PR domain containing 1 (PRDM1), a transcription factor with mainly repressor function, plays a pivotal role in orchestrating the differentiation of B cells into plasma cells.[47–49] It exerts gene repression both directly, via a histone methyltransferase domain (PR domain), and indirectly, via five zinc-finger domains that bind to the DNA and recruit histone deacetylases (HDACs) of the Groucho family.[50,51] In humans,

BLIMP1 exists in two different isoforms arising from alternate promoters: *PRDM1α* encodes the full-length BLIMP1 protein, while *PRDM1β* lacks the first 101 amino acids, resulting in loss of most of the PR domain and reduced function as a transcriptional repressor.[52] BLIMP1 orchestrates mature plasma cell differentiation by suppressing genes necessary for commitment and maintenance of B-cell identity and for cell proliferation.[53] Among the genes silenced by BLIMP1 are the B-cell surface markers *CD19*, *CD20* and *CD45*; *MIP-1β* and *CD69*, which participate in B-cell activation; the transcription factors *BCL6*, *PAX5*, *STAT6*, and several B-cell-receptor signaling cascade molecules such as *SYK*, *BLNK*, *CD79a*, *PKCβ*, and *LYN*.[54,55] *PAX5* is fundamental for commitment to B-cell lineage during early hematopoiesis and its suppression is necessary for B cells to differentiate into plasma cells.[56–58] Among its functions, PAX5 represses the transcription factor *X-binding protein-1 (XBP-1)*, the only known substrate of the endoribonuclease activity of inositol-requiring enzyme (IRE-1), one of the three sensor branches in mammalian unfolded protein response (UPR).[59,60] XBP-1 was shown to be necessary for terminal differentiation of B lymphocytes into plasma cells by inducing expression of genes involved in the process of immunoglobulin synthesis and folding.[49,61]

Class II transactivator (CTIIA), the master regulator of expression of major histocompatibility complex class II, is also a target of BLIMP1-mediated gene repression, thus accounting for the downregulation of major histocompatibility complex class II in antibody-secreting plasma cells.[62] BLIMP1 also mediates the repression of genes involved in DNA replication and repair and in cell cycle progression, including *C-MYC*, which is necessary, but does not suffice to drive the terminal differentiation of B lymphocytes.[63] In light of the spectrum of genes inhibited by *BLIMP1*, a role as a tumor suppressor gene has been recently proposed.[64]

TNFAIP3: a key molecule in terminating canonical NF-κB pathway activation

Initially identified as a gene induced by TNF-α, *TNFAIP3* (also known as *A20*) codifies for a downstream molecule in the NF-κB pathway and exerts a crucial function in the negative feedback loop that terminates NF-κB signaling.[65] Its induction is mediated by engagement of several receptors involved in inflammation and infection signaling, most prominently tumor necrosis factor receptor 1 (TNFR1), the B-cell receptor CD40, toll-like receptors (TLRs), NOD-like receptors, and the interleukin-1 receptor (IL-1R).[66]

Structurally, TNFAIP3 is an ubiquitin-editing enzyme characterized by an N-terminal deubiquitin (DUB) domain and a C terminus containing seven zinc-finger domains with direct ubiquitin binding and ubiquitin ligase (E3) activity.[67] Among the substrates of the DUB activity of TNFAIP3 is receptor-interacting protein 1 (RIP1). In response to TNF alpha signaling, RIP1 is ubiquitinated at lysine (K) 63, which results in recruitment of transforming growth factor β-activated kinase 1 (TAK1), TAK1-binding protein 2, and IκB kinase γ, also known as NEMO. This complex is responsible for the phosphorylation of NF-κB inhibitor-α, IκBα which results in its polyubiquitination via K48 and subsequent proteasomal degradation. Proteasome-mediated degradation of IκBα causes activation of the canonical NF-κB pathway. TNFAIP3 causes inhibition of the canonical NF-κB pathway by deubiquitinating RIP1, thus abolishing the recruitment of the TAK1, TAK1-binding protein 2, and NEMO complex.

The E3 ubiquitin ligase, TNF receptor-associated factor 6 (TRAF6) is also substrate of the DUB activity of A20.[68] Upon engagement of TLR4 and IL-1R, TRAF6 binds to Ubc13 (or Ubc5), an E2, ubiquitin-conjugating enzyme. This association causes TRAF6 activation via K63 autoubiquitination. Polyubiquitinated TRAF6 activates the IκB kinase complex (IKK), resulting in IκB phosphorylation, its proteasomal degradation, and activation of the canonical NF-κB pathway.[69]

The E3 domain of A20 is involved in K48-polyubiquitination of several E2 enzymes, including UBCH5 and UBC13, which promotes the activation of the signaling molecules RIP1, TRAF6, IκB kinase γ, and RIP2 via K63 ubiquitination. In a close regulatory feedback, RIP1 is also a direct target of the ubiquitin ligase activity of TNFAIP3, leading to its proteasomal degradation.[66]

TNFAIP3 appears to mediate inhibition of the NF-κB pathway also via direct binding of ubiquitin chains on IKK and TNFR1, resulting in non-catalytic interference with their signaling function. In vitro studies have shown that the zinc-finger domains 4 and 7 are responsible for this action.[70]

Given the activity of A20 in terminating NF-κB signaling, its loss of function by mutation or deletion has been advocated to play a major role in several inflammatory, autoimmune, and malignant human diseases, including WM.[65,66] In particular, TNFAIP3 is deleted in around 40% of WM patients, with 5% presenting with bi-allelic inactivation, suggesting that it has an important role as oncosuppressor in this disease.[46]

Importance of non canonical NF-κB pathway deregulation in the pathogenesis of WM: identification of mutations in tumor necrosis factor-receptor-associated factor 3 (TRAF3)

The canonical pathway of NF-κB activation involves phosphorylation of IκB by the IKK complex (composed by two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKλ), resulting in its degradation via the proteasome. Proteolysis of the inhibitory subunit relieves cytosolic sequestration of the active dimers 50/RelA and p50/c-Rel, which then translocate to the nucleus and exert their function as transcription factors. Instead, the noncanonical pathway of NF-κB activation requires proteasome-mediated proteolytic activation of the precursor protein of NF-κB2 (p100) into the p52 active component in order to allow its dimerization with RelB and thus activation of signaling.[71] Such activating cleavage occurs after IKKα-mediated phosphorylation and ubiquitination of p100, a signaling induced by NF-κB-inducing kinase (NIK). TRAF3 mediates proteasomal degradation of de novo synthesized NIK by recruiting the E3, ubiquitin ligase complex, TRAF2-cIAP (cellular inhibitor of apoptosis). In the absence of TRAF3, NIK is not targeted for degradation, thus resulting in chronic NF-κB signaling. This function has been advocated to explain the tumor suppressor role of *TRAF3*.[72] This gene is located on the long arm of chromosome 14 at cytoband 32.32. Bi-allelic deletions or inactivating mutations at this site were noticed in about 5% of WM patients in a recently published microarray-based study of 42 WM patients.[46,73]

Impact of whole genome sequencing: the identification of MYD88

Cytogenetics, FISH, and GEP provided significant information regarding the genetics of WM but did not reveal any specific molecular signature capable of distinguishing WM from other B-cell malignancies.[27] This observation suggests that SNPs, small genetic mutations, and/or epigenetic mechanisms might be responsible for the unique WM phenotype. Whole genome sequencing and Sanger sequencing revealed the presence of a recurrent, missense, single-nucleotide mutation on chromosome 3p22 in 30 patients with WM.[74]

This non-synonymous mutation causes a leucine to proline substitution in codon 265 (L265P) of the *Myeloid Differentiation Primary Response Gene 88* (*MYD88*).[74] MYD88 is believed to be a key player in the activation of the canonical NF-κB pathway, downstream of TLR and IL-1R signaling.[75,76] Upon TLR or IL-1R activation, MYD88 homodimerizes and forms a complex with interleukin-1 receptor-associated kinase (IRAK) 4, resulting in activation of IRAK1 and IRAK2.[77] The former is responsible for the activation of TRAF6, which cooperates with TAK1 in phosphorylating and activating the IKK complex.[77,78] The latter targets IκB for degradation, thus leading to canonical NF-κB signaling.

The L265P MYD88 mutation was first reported in diffuse large B-cell lymphoma and has recently been shown to be present in over 90% of WM patients when assessed via whole genome sequencing, Sanger sequencing, or allele-specific polymerase chain reaction (AS-PCR).[74,79,80] Given the role of MYD88 as an amplificator and transducer molecule downstream of TLR and IL-1R, the L265P mutation is believed to result in gain of function and chronic activation of the canonical NF-κB pathway.

While L265P MYD88 was detected in only 10%–12% of IgM-MGUS via whole genome sequencing, in a recent study that utilized AS-PCR, it was found present in around 50% of 24 patients analyzed. This observation suggests that L265P MYD88 mutation might be an early oncogenic or driving mutation in the evolution of IgM-MGUS to WM.[80] Interestingly, the three IgM-MGUS patients who progressed to WM in the same study presented a high level of expression of L265P MYD88 mutation when assessed by real-time polymerase chain reaction and were also all positive for mutation on sequencing. These data suggest a potential prognostic role of L265P MYD88 in stratifying IgM-MGUS patients for risk of progression to WM.

In WM patients, the presence of L265P MYD88 correlated with a higher burden of bone marrow involvement compared with patients who did not carry the mutation and response to treatment resulted in decreased levels of L265P MYD88 as assessed by real-time AS-PCR.[80] Moreover, when combined with immunoglobulin heavy chain variable region (IGHV) mutation status, the L265P MYD88 mutation appears to outline a unique genetic signature that distinguishes WM from other similar B-cell malignancies.[81] Taken together, these data suggest that L265P MYD88 may be used as a molecular marker for diagnosis, risk stratification, and monitoring of disease response to treatment in patients with WM. Given its putative role in the uncontrolled activation of the canonical NF-κB pathway, L265P MYD88 is an extremely attractive target for pharmacologic inhibition in WM.

Epigenetics and miRs: a different way of targeting genes in malignancy

"Epigenetics" (from the ancient Greek "epi," meaning above, and "genesis," meaning origin) is the study of heritable changes in gene expression caused by mechanisms other than modifications in the nucleotide sequence. DNA methylation, acetylation, and miRs are some of the major mechanisms to modulate gene expression and cellular phenotype without altering gene sequence.

miRs are noncoding RNA oligonucleotides about 20 bases long that exert their function by binding to newly transcribed, target messenger RNAs, thus inhibiting their translation. Each miR can interfere with the translation of a pool of genes, rather than a single transcript, thus allowing modulation of multiple targets at once. Recently, miRs have been reported to play a major role as either oncogenes or oncosuppressors in both hematologic and solid malignancies, depending on the function of their target genes.[82]

Via comparative analysis of miR expression in WM cells and their normal counterparts (CD19+ cells isolated from the peripheral blood and bone marrow of healthy donors), a distinct pattern has emerged that distinguishes the former from the latter. WM cells showed upregulation of miRs 155, 184, 206, 363, 494, and 542-3p and downregulation of miR-9.[83] In patients, this signature miR profile correlated with an elevated IPSS score at diagnosis, thus suggesting a role for it as a prognostic factor. Moreover, in vitro treatment with anti-WM drugs such as rituximab, perifosine, and bortezomib resulted in decreased level of the upregulated miRs and increased miR-9 level, suggesting miRs have a primary etiopathologic role and potential function as a predictive factor of response to therapy.

miR-155 was shown to be overexpressed in several cancers of B-cell origin and to act as an oncogene by deregulating NF-κB, PI3K-AKT and MAPK/ERK signaling pathways.[84–87] miR-155 was also shown to be involved in WM cell adhesion to fibronectin and in chemotaxis in response to stromal-derived factor 1 (SDF-1), thus suggesting a key role in mediating the localization and retention of cancer cells into the nurturing bone marrow niche. To further substantiate this function, miR-155 knockdown WM cells were investigated and showed impaired homing and trafficking when injected into mice.[88] Further, these animals were noticed to have prolonged survival when compared with animals injected with wild-type cells. GEP also showed decreased expression of cyclins D1, D2, D3, and E; of cyclin-dependent kinase-2, -4, and -6; and increased expression of the cyclin-dependent kinase inhibitors p18, p19, p21, and p27 which altogether resulted in G1-G0 arrest and decreased progression to S phase. miR-155 knockdown in WM cells also results in decreased Mdm2 expression with consequent increased p53 level and activity.[89]

miR-206 and miR-9 have an amplified epigenetic function by targeting histone deacetylase (HDAC) and histone acetyltransferase (HAT), respectively. In particular, overexpression of miR-206 in WM results in increased levels of HDAC-2, -4, -5, -6, -8 and -9 while the downregulation of miR-9 mediates decreased expression of HAT1, 2 and 3. Overall, the net balance of their functions is increased histone acetylation leading to enhanced gene transcription.[90]

Deletion of 13q14 was reported in about 10%–13% of patients with WM.[27,31] The minimal deleted region of this genetic abnormality includes the miR genes *MIRN15A* and *MIRN16-1*, which have been previously reported to be downregulated or lost in about 70% of CLL patients.[91] Both miRs are believed to act as tumor suppressors by inhibiting the transcription of a pool of mRNAs encoding for proteins involved in cell cycle, proliferation, and anti-apoptosis, such as BCL2.[92] Previously regarded as a sign of disease progression and aggressive biologic behavior, deletion 13q was recently reported to have no prognostic value in WM.[31]

Conclusion

Genetic studies in WM have been historically hampered by the low proliferation rate of tumor cells. With the evolution of methods and technologies in the biomedical field, the past 15 years have witnessed an exponential growth in knowledge about gene aberrations in WM. This increased fund of information is helping to delineate a genomic signature that will serve not only as a risk stratifying measure for WM patients but also as a tool to better understand the etiopathogenic bases of the disease.

From standard cytogenetics to gene array and whole genome sequencing, aberrations in the NF-κB pathway have been proven to play a central role as a pathogenic mechanism in WM, opening the way toward the design of target therapies. WM cells have also been shown to harbor mutations in genes coding for miRs, HATs, and HDACs, with a net result of generalized increase in gene transcription. These data have helped expand our understanding of the role of epigenetics in tumorigenesis and paved the way for the design of therapies to target miRs as a tool to affect multiple potential targets simultaneously.

On the basis of the recently gathered genetic data, in particular the highly represented L265P MYD88 mutation,

the scientific community looks forward to the identification of the molecular mechanisms of WM pathogenesis and the development of specific inhibitors against this newly identified molecular target.

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Disclosure

The authors declare no conflicts of interest in this work.

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