Waldenström’s macroglobulinemia: Two malignant clones in a monoclonal disease? Molecular background and clinical reflection

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Funding information
The Ministry of Health of the Czech Republic, Grant/Award Number: RVO - FNOs/2015; The Ministry of Education, Youth and Sports, Grant/Award Number: SGS09/LF/2016-2017; Institutional Development Plan of University of Ostrava, Grant/Award Number: IRP201550

Abstract
Waldenström’s macroglobulinemia (WM) is a complex disease characterized by apparent morphological heterogeneity within the malignant clonal cells representing a continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. At the molecular level, the neoplastic B cell–derived clone has undergone somatic hypermutation, but not isotype switching, and retains the capability of plasmacytic differentiation. Although by classical definition, WM is formed by monoclonal expansion, long-lived clonal B lymphocytes are of heterogeneous origin. Even more, according to current opinion, plasma cells also conform certain population with pathogenic and clinical significance. In this article, we review the recent advances in the WM clonal architecture, briefly describe B-cell development during which the molecular changes lead to the malignant transformation and mainly focus on differences between two principal B-lineage clones, including analysis of their genome and transcriptome profiles, as well as immunophenotype features. We assume that the correct identification of a number of specific immunophenotypic molecular and expression alterations leading to proper aberrant clone detection can help to guide patient monitoring throughout treatment and successfully implement therapy strategies directed against both B- and plasma cell tumor WM clones.

KEYWORDS
B-Lymphocytes, Clone Cells, Immunophenotyping, Waldenstrom Macroglobulinemia

1 | INTRODUCTION

Waldenström’s macroglobulinemia (WM) is a B-cell lymphoproliferative malignancy characterized by the high level of monoclonal immunoglobulin M (IgM) paraprotein in blood serum. It is associated with the bone marrow infiltration by small lymphoma cells capable of malignant proliferation.

This neoplasm is rare, indolent in nature, but, despite advances in treatment, currently is still incurable. Although our understanding of the disease biology has improved substantially, no consensus in phenotypic characterization of Waldenström’s clone has been reached thus far.1–4 Almost every case of Waldenström’s macroglobulinemia is associated with a somatic mutation L265P in MYD88.5 Significantly higher risk of progression from the IgM monoclonal gammopathy of
undetermined significance (IgM-MGUS) to WM for patients with mutated MYD88 gene suggests that this mutation plays a central role in malignant development as an early oncogenic event. Second most prevalent mutation in chemokine receptor CXCR4 gene also has clinical implications, and the mutated protein represents modulator of drug resistance in WM.

Research of last decades demonstrated that the clonal lymphoplasmacytic cell phenotype in WM corresponds to the late stage of B-cell differentiation and probably is derived from the IgM producing memory B cells that have undergone somatic hypermutation, but not isotype switching. Despite being traditionally considered of monoclonal origin, recent findings proved that WM consists of two clonal populations evolved from a common ancestor. Such clonal heterogeneity has been also observed in another monoclonal gammopathy, multiple myeloma.

In this article, we review the recent advances in understanding the WM clonal architecture and mainly focus on differences in genome and transcriptome profiles, as well as in immunophenotype between two founder B-lineage malignant clones. Finally, we discuss the probable contributions of each clone in the pathogenesis and possible treatment strategies.

2 | REGULATION OF B-CELL DEVELOPMENT: WHERE IS THE MISTAKE?

Tumor cells of Waldenström’s macroglobulinemia derive from B lymphocyte during its development and show a wide morphological heterogeneity. In general, the process of B lymphocyte development is regulated by several transcription factors and is accompanied by multiple changes of the cell immunophenotype. Different stages of B-cell development from early lymphocyte progenitor (pro-B cell) to antibody-secreting plasma cell including major immunophenotypic markers and regulators of differentiation are summarized in Figure 1. Differentiation of B-cell precursors from hematopoietic stem cells (HSC) to naïve mature B lymphocytes in the bone marrow is the first step followed by maturation to the memory/effector cells in the secondary lymphoid tissues, bone marrow and spleen. Each specific B-cell subset requires special microenvironment which is mainly shaped by T cells and other leukocytes. Bone marrow or fetal liver niches provide cytokines and chemokines that activate signaling pathways regulating expression of transcription factors which in turn control genes governing the accurate B-cell development. Early B cells undergo V(D)J immunoglobulin gene rearrangement preceding immunoglobulin expression. This process results in more than $10^{12}$ different immunoglobulin gene sequences, meaning that each B cell is unique.

Two additional processes introduce more variability into the immunoglobulin genes during maturation in the germinal center (GC). The first one is called somatic hypermutation (SHM) and causes point mutations in the already rearranged genes. The second one is class switch recombination (CSR) which results in exchange of immunoglobulin class IgM into IgG, IgD, or IgA. Clonal malignant cells come from one clone of the progenitor; therefore, they have the same gene rearrangement of the V(D)J locus. Deregulation of B-cell development and mutations in various genes in mature B cells lead to malignant

![FIGURE 1](image-url)
transformation and Waldenström’s macroglobulinemia manifestation. Pathological WM cell population is represented by aberrant cells of mature stages of B-cell development as germinal center B cells, plasmablasts, and plasma or memory cells (Figure 1).

3 | WM BIOLOGY: WHAT WE KNOW ABOUT THE TUMOR MASS

Our understanding of the pathogenesis of WM has improved considerably with the recent discovery of commonly recurring, acquired somatic point mutations. The presence of MYD88L265P mutation in has been described with 80%-100% and 50%-80% prevalence in WM and IgM-MGUS patients, respectively.6,17-21 The presence of MYD88L265P in the precancerosis IgM-MGUS suggests that this mutation represents an early oncogenic event in WM pathogenesis.22 Gene MYD88 encodes a 33 kDa adaptor protein containing an N-terminal death domain (DD) and a C-terminal Toll/interleukin-1 receptor (TIR) domain separated by a short linker region.23 The protein serves as an anchor to recruit signaling proteins to the (TIR/IL-1R) receptors.24 Studies of signaling pathways demonstrated that the mutant protein encoded by the MYD88L265P triggers tumor growth through the activation of nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) by Bruton’s tyrosine kinase and IRAK1/IRAK4.25,26 The frequency of MYD88L265P mutation is much lower in other related indolent B-cell lymphomas such as splenic marginal zone B-cell lymphoma, chronic lymphocytic leukemia (<10%), and multiple myeloma (0%). Further studies on this mutation could reveal a very useful diagnostic marker to distinguish WM from other B-cell–related disorders.5

There are over 30 different mutations in the C-terminus of the CXCR4 gene identified in WM patients. The most common type is S338X nonsense mutation that causes increased activation of the Akt, ERK, BTK pathways resulting in augmented migration, adhesion, growth and survival of cells in WM.7,27 Mutation in CXCR4 gene also confers primary resistance to ibritinib treatment.28

Analyses of the gene-expression profile of the two cell population (BC and PC) in WM compared to the normal, chronic lymphocytic leukemia (CLL), and MM cells revealed that WM cells cluster next to their respective normal cell counterparts. It seems to be higher similarity level between WM clonal cells and normal cells of WM patients than between aberrant cells and normal cells of different malignancies (CLL or MM).29 To find out the mechanism of the Waldenström’s macroglobulinemia progress, the proteomic analysis comparing WM and healthy donors’ bone marrow cells has been performed.30 The data showed >2-fold up-regulation of the Ras-related proteins Rab4 and p62DOK, Rho-related proteins (CDC42GAP and ROKα), and other proteins, such as SNX-1, Roaz, and FAS in 60% of WM samples compared to healthy individuals. When compared CD19+ and CD138+ cells of the WM patients, both cell types equally expressed upregulated proteins mentioned above.30

Circulating serum microRNA (miRNA) profile that potentially might be used to distinguish WM from IgM-MGUS, IgM-MM, and healthy individuals has been studied. Expression of the serum miR-320a and miR-320b was found to be significantly reduced in WM compared to IgM-MGUS (P < .05), IgM-MM (P < .05), and healthy individuals (P < 0.03). In WM, level of miR-320a was negatively associated with IgG2 microglobulin level and lymphoplasmacytoid cells infiltration in the bone marrow. Accordingly, high levels of miR-320a and miR-320b were observed in CD19+ mononuclear cell fractions compared to CD19+ cells.31

4 | THE TUMOR CONTENT: HETEROGENEITY CAUSES COMPLEXITY

Malignant cells of typical WM patient morphologically represent a continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells.4 These tumor cells derived from a pool of heterogeneous precursor cells, and thus, different subtypes of WM cells have originated from the different initial B cells and display morphological heterogeneity.22 Tracing the cell of origin is one of the major directions of the WM research, as its identification would allow us to understand the development of the disease and to uncover potential therapeutical targets. Several cell types have been proposed as potential WM precursors. They are represented by the CD27+/IgM+ memory cells33 and postgerminal center CD20+/IgM+/IgD+ cells,34 which possibly have undergone processes of somatic hypermutation but failed in class switch recombination.35

Analysis of the immunoglobulin (V)DJ regions that identify the WM malignant clone indicated that these cells express somatically mutated variable regions.10,36,37 Several studies demonstrated monoclonal origin of the different cell clones defined by different V(D)J rearrangements.29,38 On the other hand, biclonality in WM patients has also been documented,10 and at least in 20% of WM patients, two clonotypic sequences were detected.34 The study by Kriangkum et al33,34 suggested that the clones were derived from distinct parental B cell with different V(D)J rearrangements and different tissue localization. Interestingly, the clinical characteristics of the patients with evidence of biclonal malignant populations coincided with the characteristics of the patients with only one monoclonal cell population. The parallel coexistence of one hypermutated and one germ line clone suggests that malignant transformation events may occur at different stages of B-cell differentiation in WM.34

Thus, question of clonality in WM patients is very important. There were detected different cell clones in Waldenström’s macroglobulinemia patients ranging from B lymphocytes to plasma cells and showing different gene-expression profiles.29 Several studies detected differences in immunophenotype between present cell clones.1,2,29 Nevertheless, the correct discrimination and immunophenotyping of the clones remain controversial.

5 | MOLECULAR DIVERSITY AND CLONE FORMATION IN WM: LET US TRY TO UNDERSTAND

Clinical significance of clonal B-cell and plasma cell population in hematological malignancies is often unclear. Moreover, relationship between these two populations could be not evident, especially when
both express the same immunoglobulin light chain. Comparison of related and unrelated B-cell and plasma cell clonal population showed differential surface marker expression profile. 

6 | WM B-CELL CLONE IMMUNOPHENOTYPE

The relative percentages of B lymphocytes and plasma cells in bone marrow infiltrates vary considerably at the time of diagnosis, although immunohistochemical analyses shown that B lymphocytes usually predominate over plasma cells. Scheme of differences in immunophenotype of normal and malignant WM cells is presented in Figure 2.

Clonal lymphocytes in WM are IgM\(^+/\)CD19\(^+/\)CD20\(^+/\)CD22\(^+\) and expressed surface light chains of monoclonal origin (Figure 3). However, the intensity of CD19 and CD20 expression is lower in comparison with normal mature B lymphocytes.\(^2,41\) WM development is consistent with a progressive accumulation of light-chain restricted mature B cells up to the stage of a lymphoplasmacytic cell, which show a characteristic Waldenström's phenotype: SmlgM\(^+\)/CD22\(^-\)/CD25\(^-\). In general, WM-BC immunophenotype was described as CD22\(^lo\)/CD23\(^-\)/CD25\(^+\)/CD27\(^+\)/SmlgM that differed from other B-cell chronic lymphocytic leukemia and mantle cell lymphoma (typically CD5\(^hi\)) or follicular lymphoma (usually CD10\(^+\)) can be observed.\(^1,44-48\)

Intensity of CD38 antigen expression is heterogeneous and lower than normally found on B cells and PC progenitors. Overall, it is present in a half of WM cases. On the other hand, CD45 is expressed in all samples and is similar to normal mature B cells.\(^2\)

7 | WM B-CELL CLONE MOLECULAR BIOLOGY: SIMILARITY TO NORMAL B CELLS

7.1 | WM-BC vs Normal BC

The majority of gene-expression studies focused on WM pathological differences included healthy cells as reference. The comparative analyses of the gene-expression profile of WM-BC vs normal BC identified a set of 171 genes differentially expressed between the two groups (109 genes were downregulated in WM-BC, and 62 were upregulated).\(^29\) The main transcriptomic changes characteristic for WM-BC and WM-PC are summarized in Table 1.

Upregulation of interleukin 6 (IL6) in these clonal cells\(^29,49\) is in concordance with data showing high serum IL6 concentration in WM patients.\(^50\) Other genes showing the highest differences are calcium binding protein (S100A8), the oncogene FOSB, and hematopoietic cell kinase (HCK).\(^29\) All these genes are involved in molecular transport and cellular proliferation. Also CD1C expression was more than 2-fold higher in most WM compared to normal B cells.\(^49\) A group of overexpressed genes includes JUN and BATF, both involved in proliferation, transformation, and cell death.\(^29\) Downregulation of interleukin 4 receptor (IL4R) is characteristic for WM-BC.\(^29\) While IL4R plays an important role in development of CLL-B cells,\(^51\) its expression seems not to be crucial for WM-BC development. The second gene with significantly lower expression is BACH2,\(^29\) and it was proved as negative expression of CD5, CD10, CD11c, or CD103.\(^1\) Monoclonal expression of surface light chains trends to predominance of slgκ vs. slgλ, with ration 5:1.\(^2\) Nevertheless, Waldenström's clone showed some degree of phenotypic overlap with marginal zone lymphoma, characterized by dimmer expression of surface IgM, heterogeneous CD25, and bright staining for CD22.\(^42\) Moreover, in some cases CD5, CD10 or CD23 could be expressed in WM B-cell clone as well.\(^2,43\)

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FIGURE 2 Immunophenotype of normal and malignant cells in WM. The scheme represents the most abundant immunophenotype of each cell type. Absence of surface marker is depicted as -. Presence of surface marker is depicted as +. [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Subpopulations of B cells in WM. Immature and residual polyclonal B cells form minor subpopulations in WM while clonal WM-related B cells CD19\(^lo\)/CD22\(^-\)/CD25\(^+\)/FMC7\(^-\) dominate the sample of bone marrow. Analyses were made by flowcytometer FACS Cantoll (BectonDickinson, Franklin Lakes, New Jersey, USA) and reanalysis by software Infinicyt (Cytognos) [Colour figure can be viewed at wileyonlinelibrary.com]
essential for class-switched plasma cell but not for development of IgM plasma cells. Bach2 downregulation in WM-BC is confirmed by data in study Paiva et al. where WM-BC were compared to healthy CD22+ 25− B cells.

No difference in gene expression was observed between clonal B cells from IgM-MGUS and WM patients. On the other hand, expression of 327 genes was deregulated when normal bone marrow mature B lymphocyte with CD22+CD25− phenotype was specifically compared to WM-BC. L2RA (CD25) and CD27 were overexpressed in WM-BC, while group of downregulated genes contains CD200 and LAIR1. Clinical significance of the expression of leukocyte-associated immunoglobulin-like receptor-1 (LAIR1, CD305), an inhibitor of B-cell receptor-mediated signaling, has been reported in chronic lymphocytic leukemia. Downregulation of LAIR1 was observed in high-risk

### TABLE 1
Transcriptomic profile of Waldenström’s macroglobulinemia BC and PC: symbol ↑ stands for overexpression of gene, symbol ↓ stands for downregulation of gene in target population of BC-WM or PC-WM

| Gene symbol | Gene full name | Function | Cell population |
|-------------|----------------|----------|-----------------|
| IL6         | Interleukin 6  | Active in inflammation and B-cell maturation | ↑WM-BC |
| S100A8      | Calcium-binding protein | Regulation of inflammatory processes and immune response | ↑WM-BC |
| FOSB        | FBJ murine osteosarcoma viral oncogene homolog B | Regulators of cell proliferation, differentiation, and transformation | ↑WM-BC |
| HCK         | Hematopoietic cell kinase | Expressed in cells of the myeloid and B-lymphocyte cell lineages, enhances cell proliferation | ↑WM-BC |
| JUN         | Jun proto-oncogene | Proliferation, transformation and cell death | ↑WM-BC |
| BATF        | Basic leucine zipper ATF-like T transcription factor | Proliferation, transformation and cell death | ↑WM-BC |
| CD1C        | CD1c molecule | Presentation of primarily lipid and glycolipid antigens of self or microbial origin to T cells | ↑WM-BC |
| ATXN1       | Ataxin 1       | Repress Notch signaling, associated with neurodegenerative disease | ↓WM-BC |
| LEF1        | Lymphoid enhancer-binding factor 1 | Mediators of WNT signals-transduction pathway | ↓WM-BC |
| FMOD        | Fibromodulin | Assembly of the collagen fibers of the extracellular matrix | ↓WM-BC |
| CCR7        | Chemokine receptor 7 | Regulate trafficking of normal B, T, and dendritic cells into lymph node | ↓WM-BC |
| IL4R        | Interleukin 4 receptor | B-cell development | ↓WM-BC |
| BACH2       | BTB domain and CNC homolog 2 | Essential for class-switched plasma cell | ↓WM-BC |
| MS4A3       | Membrane-spanning 4-domains, subfamily A, member 3 | Hematopoietic modulator for the G1-S cell cycle transition | ↑WM-PC |
| MYB         | V-myb myeloblastosis viral oncogene homolog | Oncogene, essential role in the regulation of hematopoiesis | ↑WM-PC |
| PAX5        | Paired box 5 | Regulation of plasma cell differentiation | ↑WM-PC |
| EBI2        | Epstein-Barr virus induced gene 2 | Regulation of differentiation of germinal center B cells | ↑WM-PC |
| IGHM, IGKV1–5, IGLC2, IGKC | Ig molecules | Ig molecules | ↑WM-PC |
| TNFRSF7/CD27 | Tumor necrosis factor receptor superfamily, member 7 | Regulating B-cell activation and immunoglobulin synthesis | ↑WM-PC |
| IRF4/MUM1   | Interferon regulatory factor 4/multiple myeloma oncogene 1 | Controls plasma cell differentiation and class-switch recombination | ↓WM-PC |
| BLIMP1      | PR domain zinc finger protein 1 | Transcription factor necessary for the generation of plasma cells | ↓WM-PC |
| SEPP1       | Selenoprotein P, plasma, 1 | Oxidative stress response | ↓WM-PC |
| COX7C       | Cytochrome c oxidase subunit VIIc | Mitochondrial function | ↓WM-PC |
| ATP6V0E     | ATPase, H+ transporting, lysosomal 9 kDa, V0 subunit e | Transmembrane transport | ↓WM-PC |
| CANX        | Calnexin | Facilitating protein folding and assembly | ↓WM-PC |
| PSENEN      | Presenilin enhancer 2 homolog | Processing of beta-amyloid protein precursor | ↓WM-PC |
| DKK1        | Dickkopf-related protein 1 | Fundamental regulator of MM bone disease | ↓WM-PC |
| FRZB        | Frizzled-related protein gene | Regulation of bone development | ↓WM-PC |
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was studied in BM CD19\textsuperscript{+} isolated WM cells and compared with CD19\textsuperscript{+} cells isolated from BM and peripheral blood mononuclear cells (PBMCs) of healthy donors, and in WM cell line BCWM1. The analysis showed that six genes, miRNA-363, 206, 494, 155, 184 and 542-3p, demonstrated increased expression in WM patients. On the other hand, miRNA-9 showed decreased expression in WM patients. The molecular signature of these 7 miRNAs significantly correlated with prognostic factors including age, IgJ2-microglobulin, Hb, and IgM levels, and platelet count, according to the IPSS (International Prognostic Staging System) for WM.55 Another study analyzing transcriptional profile of WM cells and classification of patients according to mutational profile of MYD88 and CXCR4 suggested similarities of patients with the presence of CXCR4\textsuperscript{WHIM} and healthy memory B and circulating B cells. MYD88\textsuperscript{L265P/CXCR4\textsuperscript{WHIM}} patients also show reduced differentiation and accompanying lower expression of genes linked to transition of cells from memory B cells to plasmablasts and plasma cells. These patients followed a pattern resembling healthy peripheral B cells, despite the presence of MYD88\textsuperscript{L265P} mutation. However, there is a controversy in right selection of population for gene-expression analyses, because whole population of CD19\textsuperscript{+} cells was used for each patient (MYD88\textsuperscript{L265P}CXCR4\textsuperscript{WHIM}, MYD88\textsuperscript{L265P}CXCR4\textsuperscript{WT}, or MYD88\textsuperscript{WT/CXCR4\textsuperscript{WT}}); therefore, analyzed population contains not only WM cells but also healthy CD19\textsuperscript{+} cells; moreover, infiltration of malignant cell in each samples may differs as well gene expression may differ.56

7.2 | WM-BC vs other BC malignancies

The comparison of clonal B cells from WM patients with other B-cell malignancies was focused on IgM-MGUS, MM, CLL, and acute lymphoblastic leukemia (ALL).29,39,49,57 The group of genes significantly down-regulated in WM-BC includes previously mentioned IL4R and BACH2 along with ATXN1, LEF1, FMOD, and CCR7.59 One of the latter, lymphoid enhancer-binding factor 1 (LEF1), has significantly lower expression in WM-BC than in CLL-BC. This transcription factor is involved in WNT/\beta-catenin pathway and is highly expressed in immature stages of normal B lymphocytes but is dramatically and rapidly downregulated in mature B cells.58 Fibromodulin (FMOD) is a small proteoglycan and regulates another cancer-associated mechanism involved in TGF-\beta activity.59 Chemokine receptor 7 (CCR7) is known to regulate trafficking of normal B, T, and dendritic cells into lymph node.60

On the other hand, no significant changes were detected in expression of PAX5 when compared CD22\textsuperscript{+} cell in WM, CLL patients, and normal B cells.39 Interleukin 6 (IL6) and CD1C expression were more than 2-fold higher in most WM compared to CLL and normal B cells.

Unfortunately, there remains a controversy about the correct detection of different clones of WM leading to different and sometimes contradictory results. Some studies used only CD19 59,57 or CD22 39 and CD138 markers to distinguish different clones. It is worth mentioning that in these studies,59,57 different magnetic cell sorting strategies were used: CD19\textsuperscript{+}/CD138\textsuperscript{+} concurrent but not sequentially selected BM cells; CD19\textsuperscript{+} and CD138\textsuperscript{+} separately selected BM cells. Thus, revealed findings mostly represent changes in whole tumor mass, but not in each single WM-BC or -PC clones. For example, when CD19\textsuperscript{+} and CD138\textsuperscript{+} cells of WM vs. IgM-MGUS were compared, 66 differentially expressed genes were identified by microarray.57 On the other hand, when the same group used more complex immunophenotyping CD45\textsuperscript{+}/CD19\textsuperscript{+}/LAIR-1\textsuperscript{-}/CD27\textsuperscript{dim}/IgM\textsuperscript{-}/CD22\textsuperscript{dim}/CD25\textsuperscript{-} for WM-BC clone, no significant differences of expression between WM and IgM-MGUS were found,61 which is in accordance with other study comparing gene-expression profile of WM and IgM-MGUS B cells.53

Thus, findings mentioned above suggest that gene-expression profile of WM-BC is more similar to normal B cells than any other compared malignancy.

8 | WM PLASMA CELL CLONE IMMUNOPHENOTYPE

WM-PC compared to MM-PC show higher expression of CD19 and CD45. WM-PC are small based on forward scatter, showed dim expression of CD138, and slightly diminished expression of CD38. This characteristic pattern confirms the evidence of continuum with the B-cell component. Low-grade B-cell lymphoma with plasmacytic differentiation expresses significantly higher level of CD19 on clonal plasma cells contrary to only 10% evidence of CD19 in plasma cell proliferative disorders.40

Plasma cell clone in WM differs from typical PC immunophenotype of non-IgM multiple myeloma and non-IgM-MGUS. In contrast to IgM-MGUS, WM patients show a higher frequency of non-myelomatous, phenotypically almost normal, but usually clonal PC, CD19\textsuperscript{-}/CD20\textsuperscript{-}/SmIgM/CD45\textsuperscript{-}/CD56\textsuperscript{−} PC (Figure 4). Moreover, symptomatic WM versus IgM-MGUS patients show complete lack of reactivity for CD56 on bone marrow (BM) PC.1

The overall percentage of BM PC remained stable across disease development; however, within the PC compartment, there was a progressive increase in the proportion of light-chain-isotype-positive PC during MW development. Notably, the extent of clonal involvement of the BM PC compartment (but not that of mature B cells) directly correlated with the serum concentration of the IgM paraprotein.62 Nevertheless, low Kendall’s tau coefficient (0.231) did not give possibility clearly define impact of WM-PC burden in IgM paraprotein production.

Overall, these results suggest that during the transition from IgM-MGUS to smoldering and symptomatic WM (in which the majority of PC are light-chain restricted), the PC compartment is enriched in clonal PC displaying an immature/plasmablastic phenotype with restricted isotype of both the Ig heavy and light chains.

Moreover, clonal B cells with intermediate light scatter characteristics between those of small B lymphocytes and PC corresponding to lymphoplasmacytoid cells were identified in 40% of all WM patients. This subpopulation shows high expression of CD38, on the other hand lacked CD138 on the surface and is characterized by CD19, CD20, and FMC7 expression.2

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9 | WM-PC CLONE MOLECULAR BIOLOGY: INCOMPLETE MATURATION OF PC

9.1 | WM-PC vs normal PC

Origin of malignant clone in WM is a B cell that has undergone somatic hypermutation and affinity maturation but not class switching, a developmental stage between late germinal center B-cell and plasma cell. WM-PC retain intermediate gene-expression profile between clonal BL and PC as revealed by analysis where expression of 37 genes, including genes involved in plasma cell differentiation, supported such status.29 PAX5, whose transcription must be repressed to allow PC differentiation, had significantly higher expression levels in WM-PC than in normal plasma cells (N-PC). By contrast, BLIMP1 and IRF4 genes, which have an essential role in PC differentiation, had significantly lower expression levels in WM-PC than in N-PC.29,39

When comparing gene expression of WM-PC and N-PC, only 13 genes were significantly deregulated. Interestingly, that two members of the CD20 family MS4A3 and the oncogene MYB and also the Epstein-Barr virus-induced gene 2 (EBI2) showed a higher expression in WM-PC than in N-PC.29

9.2 | WM-PC vs other PC malignancies

Multiple myeloma,29,49 marginal zone lymphoma,39 and IgM-MGUS57 were involved in studies comparing differences in gene expression in WM-PC clone with other malignancies.

Comparison of nuclear proteins expression in CD138⁺ cells from WM patients (WM-PC) vs plasma cell myeloma and marginal zone lymphoma shows in WM-PC significantly higher expression of PAX5, regulatory element maintaining B-cell identity. A large number of cells with lymphocyte morphology and plasma cells were also found to be CD138 and PAX5 double positive in WM.39 On the contrary, MUM1 was downregulated in WM-PC. Expression of MUM1 leads to B-cell heavy chain class switch recombination and the generation of plasma cell from germinal center and memory B cells.63,64 One of the upstream regulators of PAX5 is the transcription factor BLIMP1 regulating the development of short- and long-lived plasma cells.53 These findings suggest that components of the B-cell transcriptional program are more active in WM-PC than in other cells.29 Gene-expression profile of WM-PC is closer to characteristic of B lymphocytes than to plasma cells in other benign and malignant tissues. This supports the results of gene-expression study that claims that WM-PC retains intermediate feature between normal B lymphocyte and plasma cell.29

Genes with higher expression in WM-PC compared to MM-PC encode Ig molecules (IGHM, IGKV1-5, IGLC2, IGKC) or belong to a tumor necrosis factor receptor superfamily (TNFRSF7). The number of downregulated genes in WM-PC is significantly higher, and the genes are involved in oxidative stress response (SEPP1), mitochondrial function (COX7C), transmembrane transport (ATP6V0E), and protein processing and folding (CANX, PSENEN).29 Absent expression of DKK1 and FRZB, fundamental regulator of MM bone disease,66 in WM-PC, could explain the lack of bone marrow lesions in WM compared to MM, despite the presence of clonal PC. Group of similarly expressed genes in WM-PC and MM-PC, but downregulated in healthy plasma cells, encodes proteins responsible for molecular transport (SCP2, PRDX1, APEX1, UGP2), RNA trafficking (RAN, XPO1), RNA post-transcriptional modification, and ribosomal proteins. These findings suggest same secretory machinery in WM and MM.29

In conclusion, gene-expression profile of WM-PC supports the opinion that WM-PC result from an incomplete maturation process of clonal B lymphocyte. Transcriptomic study suggests that WM-PC retain intermediate feature between normal B lymphocyte and plasma cell.

10 | DISCUSSION

WM is a complex disease characterized by apparent morphological heterogeneity within the malignant clone. The extent to which
the diverse subtypes of lymphocytes, lymphoplasmacytic cells, and plasma cells participate in, and contribute to, the disease progression is unknown. To our opinion, impact of PC clone in Waldenström's pathogenesis and clinic is seriously underestimated by nowadays.

The clonal lymphocytes are of B-cell lineage and usually have a characteristic immunophenotype CD22^{dim*}/CD25^{+} (Figure 3), positive for monotypic surface immunoglobulin light-chain IgM, pan-B-cell antigens, and CD5{ }^{+}/CD10^{−}. For a long time, WM-B lymphocytes were considered monoclonal. Recently, number of studies documenting that WM is composed of several clonal populations has appeared. In most cases of WM also plasma cells CD38{ }^{−}/CD138{ }^{−} and negative for B-cell antigens are present (Figure 4). The molecular differences between clones require further investigation.

Analyses of gene rearrangement and IgVH sequence demonstrate the genotypic relationship between different clonal populations. It was found that in some cases, an independent clonal plasma cell population may also be "hidden" within the plasma cell population already confirmed and characterized as WM.

Distinguishing the clonal cells in WM patient and their molecular characterization is important step for treatment and subsequent detection of the residual cell clone after clone's specific therapy. Studies detecting the residual monoclonal plasma cells in WM sample after treatment by anti-B-cell-specific antibodies were published.

It was concluded that WM can persist as a pure plasma cell population after therapy which may explain remaining IgM paraproteinemia in some WM patients with no evidence of a clonal B lymphocyte population. Nevertheless, pathogenesis of the residual PC is unknown and it still remains unclear, what is the clinical manifestation of the plasma cell clone.

The clone-specific therapies which eliminate B cells are the monoclonal antibodies rituximab and newer generation ofatumumab targeting the pan-B-cell marker CD20.

Recently, also a new monoclonal antibody agent has been evaluated in II clinical trials for WM treatment—alemtuzumab (anti-CD52). As reported Owner et al, in 4 of 5 patients, alemtuzumab therapy successfully eradicated clonal B cells from the bone marrow, but residual plasma cells remained present in 2 of these patients. It seems a promising drug, as CD52 expression was demonstrable in the prevalent majority B cells of all WM cases.

Unfortunately, there is still no trial aiming to eliminate a plasma cell component of WM. The WM-PC clone, which is usually more resistant to chemotherapy, may expand and may lead to changed clinical manifestation. Thus, it is not clear, what are the adverse effects of the "isolated" BC elimination. Investigation of clonality of any residual plasma cells must be done in all cases of WM to evaluate the presence and the level of residual or persistent disease. Alternatively, the disease may transform into the different monoclonal gammopathy, for example, plasma cell dyscrasia.

A potential confounding factor in diagnosis and treatment is that the characteristics of the disease at one time point may be dramatically different from those at another one. Identification of specific immunophenotypic, molecular, and gene-expression changes may help guide patient monitoring throughout treatment and potentially identify patients with worse outcomes. Implementation of the therapy directed to both B-cell and PC tumor clones may lead to stable remission.

ACKNOWLEDGEMENTS

We sincerely thank to Vyacheslav Yurchenko for the constructive comments on the manuscript. This work was supported by Institutional Development Plan of University of Ostrava (IRP201550); The Ministry of Education, Youth and Sports (Specific university research of the Faculty of Medicine, University of Ostrava) projects SGS09/LF/2016-2017; Ministry of Health Czech Republic no. RVO—FNOs/2015.

AUTHOR’S CONTRIBUTIONS

KG, EK, JF, and ZK wrote the manuscript; LR performed FACS analysis and critically revised the manuscript; FK wrote the manuscript and conceived the topic and the structure of the manuscript; MK and RH critically revised the manuscript; TS revised the text and performed stylistic improvements of the manuscript.

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