[ABSTRACT WM1.5]

GENETICS AND GENOMICS OF WALDENSTRÖM MACROGLOBULINEMIA

R. Fonseca, E. Braggio
Mayo Clinic, Scottsdale, AZ, USA

Waldenström's macroglobulinemia (WM) is an incurable B-lymphoproliferative disorder characterized by lymphoplasmacytic differentiation and associated with monoclonal immunoglobulin M (IgM) secretion.1,2 This disease is characterized by the proliferation of the tumour clone into the bone marrow. A precise identification of the normal counterpart of the WM clonal cell has not been established, although evidences support that they resemble post-germinal centre memory B-cells that have undergone somatic hypermutation, but transform before isotype switching.3- 8 Class switch rearrangements involving the m switch region were investigated, with no rearrangements identified.9 The etiology of this syndrome is unknown. WM is believed to be predominantly a sporadic disease, however several reports have identified a familial component, suggesting the existence of a germ-line WM susceptibility gene(s).10-11

Chromosomal abnormalities in Waldenström macroglobulinemia

Karyotype abnormalities

The genetic basis of the disease is poorly understood. Limited cytogenetic studies have been performed in WM, and identification of recurrent chromosome abnormalities associated with the pathogenesis has not been very successful because normal metaphases are usually prevalent in karyotypic analysis8,12-14 The incorporation of the cIgM-FISH technique has allowed analyzing interphase nuclei, overcoming the problems associated with low tumour cell division rate. Despite the clinical difficulty to differentiate this syndrome from other B-cell lymphoproliferative disorders with monoclonal IgM, recent cyto-molecular studies suggested that WM seems to show a unique genomic profile.

Chromosome 6 deletions

The most frequently identified abnormality in WM is the deletion of the 6q arm. Karyotype studies showed this abnormality in 16% of WM patients, but we reported a rate of >50% by using cIgM-FISH.9 This deletion usually involves chromosome bands 6q21-q23, being the q23 region the most commonly deleted. Several tumour suppressor genes are localized in that chromosomal region such as *BLIMP1* and *MYB*, but an association between deletion and decrease or loss of function of any of them remains to be documented. The 6q deletion is clonally selected. It is likely a progression event since we have shown that patients with Monoclonal Gammopathy of Unknown Significance and isotype IgM (IgMMGUS) rarely if ever have 6q deletions.14 Additionally, this abnormality is uncommon in nodal lymphoplasmacytic lymphoma - LPL.15 The high prevalence of 6q deletion and its unique presence in WM, compared to nodal LPL or IgM-MGUS, suggest a differentially cytogenetic profile associated with this abnormality. The presence of 6q deletions is not known to have clinical associations, but a recent study of ours suggested that patients with 6q presented significantly higher levels of b2m (p=0.001), anemia (p=0.01) and hypoalbuminemia (p=0.001), all features associated with poor prognosis.16 Moreover, patients with 6q deletion display a shorter treatment-free survival (median of 55.2 months versus not reached in patients without the abnormality after 100 months of follow-up; p=0.03).

Other chromosome abnormalities

Unlike IgM-MGUS and IgM Multiple Myeloma (IgM-MM), translocations that involve the immunoglobulin heavy chain locus at chromosome 14 are very rare or absents in WM.9,17 Originally, it was believed that WM contained the t(9;14)(p13;q32), involving PAX5 and IgH locus.18 The t(9;14)(p13;q32) was reported in LPL with a frequency of 50%, but it seems to be restricted to cases with no detectable IgM monoclonal protein. 19 Additionally, the biological effects of up-regulated PAX5 caused by the t(9;14)(p13;q32) are not in agreement with the WM phenotype, due to PAX5 inhibits the production of the J-chain, which is essential in the IgM pentamer formation. In our cohort of WM patients we failed to detect this translocation.9 Another report, including 69 patients has confirmed our findings.20 The t(11;18)(q21;q21) translocation usually associated with a subset of marginal zone lymphomas has been reported in sporadic cases of WM(21). However, using the cIgM-FISH strategy we were unable to detect this translocation in our cohort of patients.9 Our team was also able to show that deletions of 13q14 and 17p13 are not common at the time of diagnosis, but may be observed in 15% of patients at the time of disease progression.22 Limited studies are available with regards to the ploidy status, showing that WM clonal cells are likely diploid. Our group supported this assumption in a cohort of 15 patients by using centromere probes for chromosomes 7, 9, 11, 12, 15, 17.9 These chromosomes are frequently involved in numerical gain/losses in related diseases as MM and B Chronic Lymphocytic Leukaemia – B-CLL. However, we have not identified any numerical abnormality affecting that subset of chromosomes. In a recent study, Terre and colleagues23 reported partial copy gain and trisomies of chromosome 4 in 8 of 39 patients (20%). With the incorporation of new technologies, as the array-based comparative genomic hybridization (aCGH), is becoming possible to realize a whole genome high-resolution study. Using this approach, we identified previously undetected recurrent chromosomal abnormalities such as 3p21-22 and 8p deletions, as well as 3q13-29 gains. We confirmed that ploidy status changes are not common events in WM, detecting low frequency of chromosomes 4, 7, 9, 18 trisomies and chromosome 21 monosomies. In patients with 6q and 8p deletions, we found high frequencies of 6p and 8q arm gains, respectively. Our data suggested that these gains are secondary events, because we were not able to detect these abnormalities in patients without its respective deletions. Conversely, deletions without associated arm gains were commonly observed (*manuscript in preparation*).

Gene expression profiling of WM

Use of gene expression arrays (GEP) as a marker for genomic abnormalities and subsequently as a tool for disease profiling is a powerful approach to characterize genomic changes that are responsible for disease pathogenesis in WM. We employed GEP for comparison of WM with MM, B-CLL, smouldering myeloma, MGUS and normal B and plasma cells as a means to identify differential gene expression signatures as well as deregulated genes associated with WM.24 WM was found to cluster with CLL and normal B cells following unsupervised hierarchic clustering and only a small set of genes was found to be specific of the disease. We did not identify differences in GEP of patients with and without 6q deletions. The most significantly up-regulated genes were IL-6 and genes involved in the MAPK pathway. In relation with the D-type cyclins, WM

and CLL only expressed *D3* whereas MM expressed all 3 cyclin D genes.25 Further, Gutierrez and colleagues separated WM cells into those with B- (WM-BL) and plasma cell (WM-PC) morphology for\ gene expression comparison to CLL, MM and normal individuals.26 Following unsupervised hierarchic clustering, WM-BL samples clustered with CLL while WM-PC samples were segregated with MM. Overall, the authors concluded that BL and PC from WM patients displayed differing patterns of gene expression when compared to BL and PC from CLL and MM. This study also identified up-regulation of *IL-6* in WM samples. This cytokine is currently being considered as possible therapeutic target, and also could explains the clinical observation of elevated C-reactive protein serum levels in many patients with WM. Additionally, this may be one of the many possible factors explaining anaemia in patients with WM. Finally, proteomic analysis of signalling pathways were performed in samples obtained from WM and MM patients.27 Samples from both diseases were compared before and after treatment with a proteasome inhibitor.27 While several overlaps in signalling were observed between WM and MM, the authors believed this to be due to similar pathways utilized in cell signalling for B-cell differentiation. However, after clustering analysis were performed, the authors identified groups of proteins that were expressed by either WM or MM, but not both; which indicates differences in cellular response induced by proteasome inhibitor treatment.

Conclusion

WM is an incurable late B-cell malignancy that secretes high levels of immunoglobulin M. This disorder can typically be differentiated from MM based on heavy chain isotype of antibody produced (IgM) and the lack of lytic bone lesions. While clinically similar, the cytogenetics and genomics underlying MM and WM are quite different. Gene expression profile studies have suggested that WM is more similar to CLL and normal B-cells than to MM. In contrast to MM, the genetic of WM appears to be much simpler, with less observed aneuploidy and fewer structural abnormalities, being 6q deletion the most frequently observed. Although cyto-molecular data recognized a unique genomic signature in WM, our knowledge about molecular pathways involved in the pathogenesis and disease progression is still very fragmented. Tools such as array-based comparative genomic hybridization and gene expression profile allow us to realize a high-resolution whole genome screening for abnormalities as well as to gain insights into the consequences of genomic alterations found in WM. In the future, therapeutic decisions may be based solely on data transformed from these high throughput genomic studies into practical clinical tools used for patients with WM.

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