Microarray Demonstrates Different Gene Expression Profiling Signatures Between Waldenström Macroglobulinemia and IgM Monoclonal Gammopathy of Undetermined Significance

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Abstract

Waldenström macroglobulinemia (WM) (symptomatic and indolent) and immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (IgMMGUS) can be identified based on the bone marrow (BM) infiltration and the existence of symptoms. The purpose of this study was to investigate the biological and genetic characteristics of both disorders comparing the molecular signature of WM versus IgMMGUS using microarray analysis. We investigated BM $CD19^+$ cells isolated from 21 WM patients and 10 IgMMGUS cases, and $CD138^+$ BM cells isolated from all of the WM patients and 4 of the IgMMGUS cases. Gene expression profiling of WM versus IgMMGUS $CD19^+$ cells highlighted 151 differently expressed genes and the comparison with $CD138^+$ cells demonstrated 43 differently expressed genes in WM versus IgMMGUS. Regulation of transcription, Janus kinase/signal transducer and activator of transcription, PI3K/Akt/mammalian target of rapamycin, mitogen-activated protein kinase signaling pathways are the relevant gene ontology biological processes occurring in $CD19^+$ cells, and immune response, cell activation, and signaling processes developing in $CD138^+$ cells mainly distinguish WM and IgMMGUS.

Introduction

Waldenström Macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by the accumulation of lymphoplasmacytic cells in the bone marrow (BM) and the hypersecretion of immunoglobulin M (IgM) protein.[1,2] WM cells are represented by small B lymphocytes ($CD19^+$, $CD20^+$) and differentiated plasma cells ($CD138^+$).[3]

Immunoglobulin M monoclonal gammopathy of undetermined significance (IgMMGUS) is an asymptomatic condition determined only by the presence of a monoclonal immunoglobulin (M protein).[4,5] Significantly, patients with IgMMGUS show an increased risk of developing WM.[6]

The rate of progression from IgMMGUS to WM has been noted to be 1.5%–2% a year.[7]

Until now, 2 main gene expression profiling (GEP) studies of WM have been performed: the first identified a small set of genes which characterizes WM compared with chronic lymphocytic leukemia (CLL) and multiple myeloma (MM).[8] The second GEP study demonstrated that WM B lymphocytes and plasma cells showed different expression patterns compared with the MM and CLL cell counterparts.[9]

The determination of a gene set signature and biological pathways characterizing WM and IgMMGUS, respectively, to investigate risk factors and molecular mechanisms leading IgMMGUS to progress to WM are not established so far.

In this study we performed GEP analysis of WM and IgMMGUS patients to define the molecular signature of WM B cells and plasma cells compared with their counterparts from IgMMGUS. Our aim was to identify genes differently expressed and pathways distinguishing WM from IgMMGUS to highlight the molecular and biological mechanisms typical for each disorder.

Patients and Methods

We investigated patients with WM (n = 21) and subjects affected with IgMMGUS (n = 10).

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We isolated BM CD19$^+$ cells from 21 WM patients and 10 IgMMGUS cases, and CD138$^+$ BM cells isolated from all of the WM patients and 4 of the IgMMGUS cases. BM mononuclear cells from all samples were isolated by Ficoll density gradient centrifugation at 800 rpm for 20 minutes, immediately CD19$^+$ cells were selected using MACS CD19 microbeads (Miltenyi Biotech, Bologna, Italy); afterward, CD138$^+$ cells were positively isolated from the collected CD19$^-$ cells using MACS CD138 Microbeads following the manufacturer's instructions (Miltenyi). Gene expression profiling was performed on total RNA extracted from BM CD19$^+$ and BM CD138$^+$ cells, and the concentration and quality of the RNA samples were evaluated using NanoDrop 2000 UV-vis spectrophotometer (Euroclone, Milan, Italy).

Image quantification was performed using GeneChip (Affymetrix, Santa Clara, CA) scanner and software. Preprocessing steps such as background subtraction, probe cell normalization, and expression level calculations, were performed using quantile normalization and Robust microarray analysis software.[10] Differentially expressed probe sets were selected using significant analysis of microarrays,[11] with a false discovery rate threshold equal to 5% to correct for multiple testing and an additional threshold on the absolute fold change equal to 2. Selected probes mapping on the same genes were averaged and a complete linkage hierarchical agglomerative clustering on Euclidean pair-wise distances between genes was performed.

Results and Discussion

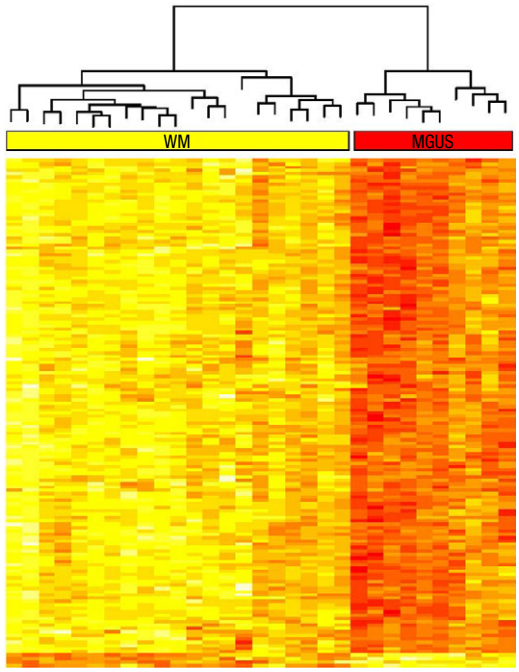
Gene expression profiles of WM BM CD19$^+$ versus IgMMGUS BM CD19$^+$ cells demonstrated that 151 genes were differently expressed (Figure 1). Functional analysis revealed as enriched the regulation of transcription: 33 genes were significantly overexpressed in WM versus IgMMGUS. BM WM CD19$^+$ cells overexpressed the following 14 zinc finger protein genes: *ZBTB40*, *ZNF83*, *ZNF137P*, *ZNF177*, *ZNF224*, *ZNF264*, *ZNF320*, *ZNF395*, *ZNF514*, *ZNF532*, *ZNF623*, *ZNF767*, *ZNF785*, and *ZNF850*. Other genes acting as regulators of transcription overexpressed in WM B cells were *HIF1AN*, *BHLHE41*, *EZH1*, *CCNL2*, *TCFL5*, *BLZF1*, *CIITA*, *PER2*, *MECP2*, *PRDM2*, and *ELP2*. Significantly, *HIF1AN* and *ELP2* play a role in the PI3K/Akt/mammalian target of rapamycin (mTOR) and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways, respectively.

Akt and mitogen-activated protein kinase (MAPK) signaling pathways were enriched by functional analysis as shown by *TNFRSF10A*, *MAP4K4*, *TNFRSF10B*, *WNK1*, *DUSP22*, and *ITPKB* genes which were over expressed in WM versus IgMMGUS B cells.

The carbohydrate metabolic process resulted to be enriched from GEP analysis and *PPP1R3E*, *AMY2B*, *AMY1C*, *AMY2A*, *AMY1B*, and *AMY1A* genes belonging to the carbohydrate metabolism were upregulated in WM CD19$^+$ cells.

A comparison between WM versus IgMMGUS BM CD138$^+$ cells demonstrated that 43 genes were differently expressed (Figure 2). *MS4A1* (CD20), *BANK1*, *GPR183*, *SWAP70*, *CD79B*, and *HLA-DOA* genes were overexpressed in WM versus IgMMGUS with high fold changes of 11.6, 9.4, 5, 5, 5, and 4, respectively. The functional analysis showed that B-cell activation and immune response are enriched

Figure 1 **Hierarchical Clustering on WM BM CD19+ and IgMMGUS BM CD19+ Patients. Heatmap of the Results of Hierarchical Clustering Performed on the 31 Patients, 21 WM and 10 IgMMGUS (MGUS in the figure), and on the 151 Genes Selected as Differentially Expressed. Clustering was Performed Using Average-Linkage Method and Euclidean Distance. Hierarchical Tree is Shown Only for the Clustering Performed on Patients**



biological processes and act differently in WM compared with IgMMGUS.

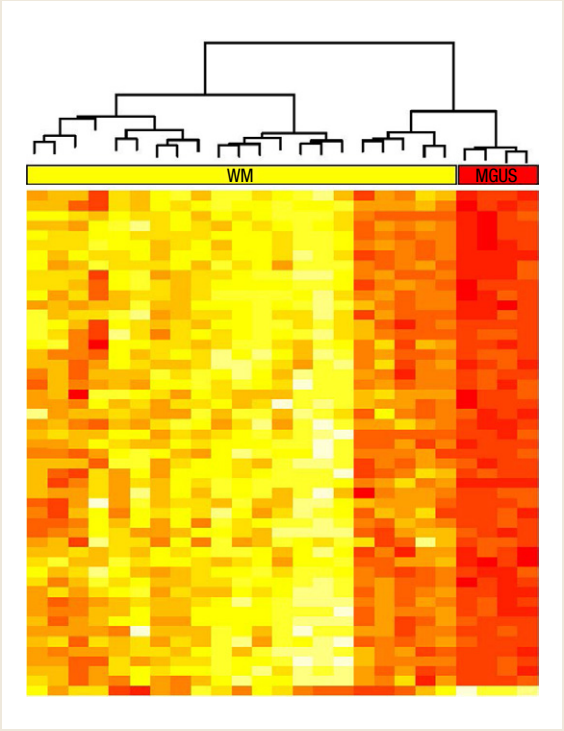
RALGPS2, *PLEKHG1*, and *ARHGAP24* genes mediating GTPase regulator activity of signal transduction were overexpressed in WM with the fold changes of 4, 10, and 4, respectively.

Gene expression profiling of BM CD138$^+$ cells also demonstrated that *FCRL2* and *FCRLA* genes involved in cell–cell signaling and cell differentiation were overexpressed in WM versus IgMMGUS, with fold changes of 4.6 and 9, respectively.

Conclusion

Gene expression profiling analysis demonstrated that BM B cells and BM plasma cells present distinct molecular signatures in the comparison between WM and IgMMGUS. It is interesting to note that the gene signature of WM versus IgMMGUS relative to BM B cells showed that all 151 genes identified as differently expressed, excluding 4 genes that were overexpressed in WM. Moreover, the regulation of transcription, JAK/STAT, PI3K/Akt/mTOR, and MAPK signaling pathways are the most relevant gene ontology biological processes occurring in BM CD19$^+$ cells reflecting different biological mechanisms in the 2 disorders.[5,12,13] These pathways play

Figure 2 Hierarchical Clustering on WM BM CD138+ and IgMMGUS BM CD138+ Patients. Heatmap of the Results of Hierarchical Clustering Performed on the 25 Patients, 21 WM and 4 IgMMGUS (MGUS in the figure), and on the 43 Genes Selected as Differentially Expressed. Clustering was Performed Using Average-Linkage Method and Euclidean Distance. Hierarchical Tree is Shown Only for the Clustering Performed on Patients



an important role in WM cells because they regulate several biological processes including cell growth, differentiation, survival, migration, and metabolism and the use of PI3K/Akt/mTOR inhibitors have already been adopted in several clinical trials.[14-16]

Gene expression profiling analysis of BM plasma cells identified a small number of genes (n = 43) with high fold changes differently expressed between WM and IgMMGUS. Immune response, cell activation, and signaling processes acting in BM $CD138^{+}$ cells mainly distinguish WM and IgMMGUS.

Future GEP studies in a large cohort of IgMMGUS cases and WM patients are necessary to highlight the biological role of the genes differently expressed in these 2 disorders and the molecular relationship between them. Moreover, the assessment of pathways and genes distinguishing WM and IgMMGUS could clarify the molecular mechanisms leading to the progression of IgMMGUS to WM in order to identify IgMMGUS patients who have a high risk of progression to WM.

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Disclosure

The authors have nothing to disclose.

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