Micro-RNAs as Epigenetic Regulators of Cytokine Signaling Pathways and the Tumor Microenvironment in Waldenström Macroglobulinemia Compared to IgM-MGUS

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INTRODUCTION

- IgM-monoclonal gammopathies consist of a broad spectrum of disease states which include monoclonal gammopathy of undetermined significance (IgM-MGUS), a benign pre-malignant condition, and Waldenström macroglobulinemia (WM), a rare non-Hodgkin lymphoma characterized by the presence of a lymphoplasmacytic infiltrate in the bone marrow associated with monoclonal immunoglobulin M (IgM).[1]
- WM is often preceded by IgM-MGUS, and the factors underlying this malignant progression remain poorly understood. Cytokines are protein factors that regulate diverse cellular processes and have been previously implicated in WM pathogenesis and their regulation may partially underlie this progression.[2]
- MicroRNAs (miRNA) are small, single-stranded, non-coding RNAs, about 22 nucleotides which perform sequence-specific regulation of target mRNA. Previous research has shown the importance of miRNAs in various aspects of non-Hodgkin lymphoma, and specifically WM pathogenesis.[3,4]
- Non-coding aspects of the genome are increasingly recognized as drivers of disease pathogenesis but remain under-explored in this disease spectrum. Epigenetic modulation of cytokines and tumor microenvironment pathways by miRNAs may provide insight into IgM-gammopathies and specifically the progression between WM and IgM-MGUS.[2]

OBJECTIVES

Identify the role of miRNAs in the regulation of cytokines and the tumor microenvironment pathways in IgM-gammopathies, specifically assessing WM compared to IgM-MGUS.

METHODS

- Sorted CD19+ and CD138+ B cells from prospectively collected bone marrow samples of patients with IgM-MGUS (n=6) and WM (n=17), along with normal controls (n=5) underwent total RNA extraction and sequencing. IgM-gammopathy was identified as a combination of both IgM-MGUS and WM.
- Profiling of miRNAs was conducted, and selection of targets of interest was performed through identifying differentially expressed miRNA with a log2 fold change (FC)>1.0 or <-1.0 and false discovery rate (FDR) <0.05.
- Next, mRNA sequencing was conducted on the same patient samples, and differential expression was performed the same way as the miRNA data to identify mRNA candidates.
- Differentially expressed miRNA/mRNA pairs with correlated biological expression, i.e. either upregulated (up) miRNA experimentally predicted to regulate downregulated (down) mRNA or vice versa, were selected.
- miRNA/mRNA pairs identified were evaluated using Ingenuity Pathway Analysis (IPA) to discover dysregulated genomic pathways. Differentially expressed miRNA and associated genes involved in altered pathways were identified.

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RESULTS

Figure 1: miRNA differential expression in IgM-Gammopathies compared to controls (A) and WM compared to IgM-MGUS (B)

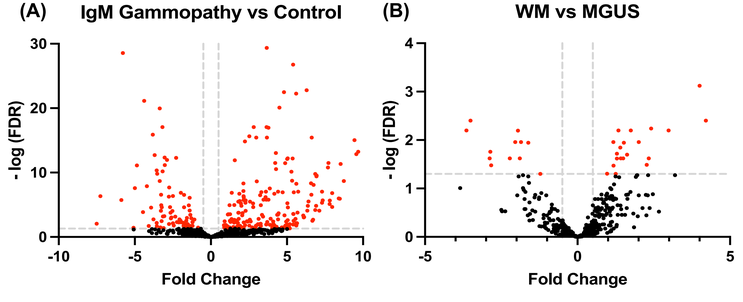


Figure 2: Differentially activated cell immune response and cytokine pathways in IgM-gammopathies compared to controls (A) and WM compared to IgM-MGUS (B)

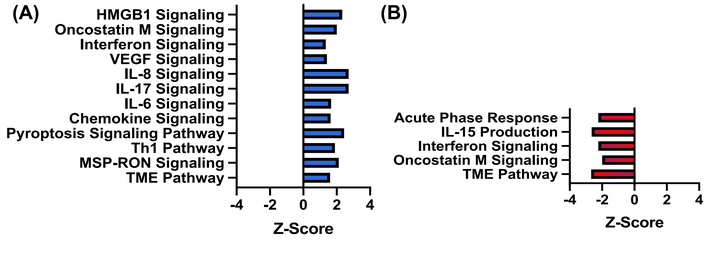
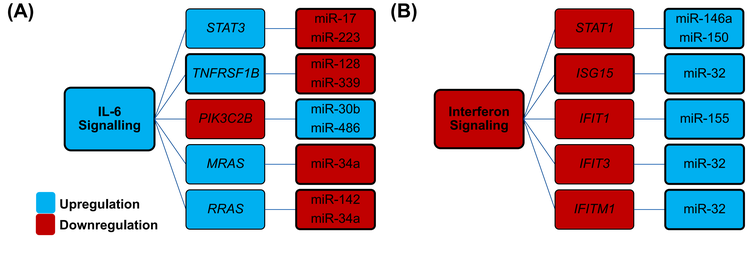


Figure 3: Assessment of the IL-6 pathway (A) in IgM-gammopathies compared to controls and interferon signaling pathway (B) in WM compared to IgM-MGUS and underling miRNA-mRNA pairs



IgM Gammopathies Compared to Controls:

- 279 miRNAs (202 upregulated/77 downregulated) were found to be differentially expressed (**Figure 1A**). Overall, there was significant miRNA-based activation of multiple cytokine pathways seen (**Figure 2A**). Many of these cytokines (ex. IL-6, IL-8, VEGF) play a known role in B-cell maturation and function, and previously multiple studies have demonstrated their importance in B-cell malignancies.[2]
- A pathway of interest was the interleukin (IL)-6 pathway which we have previously demonstrated induces IgM production in WM via the JAK/STAT pathway. Key miRNA/mRNA pairs underlying this pathway were specifically explored (**Figure 3A**). [5]
- Underlying multiple interleukin pathways, we saw implication of *STAT3*, which was upregulated, and *PIK3C2B*, which was downregulated, which are targets of miR-20B, miR-223, miR-34a and miR-491. Additionally, we observed multiple genes in the tumor necrosis (TNF) superfamily to be upregulated with targeting miRNAs which were downregulated, including miRNA-1248, miR-16, miR-18 and miR-625.

WM vs IgM-MGUS:

- 33 miRNAs (20 upregulated/13 downregulated) were found to be differentially expressed (**Figure 1B**). Pathway analysis suggested overall miRNA-based inactivation of multiple inflammatory and cytokine signaling pathways (**Figure 2B**).
- A pathway of interest was the interferon signaling pathway (**Figure 3B**). Interferons are cytokines which have an important role in regulation of cellular inflammation and immune response. Here, multiple interferon-induced genes (*IFIT1*, *IFIT3*, *IFITM1*) and signal transducer and activator of transcription 1 (*STAT1*) were downregulated. STATs play a crucial role in transmission of interferon stimulated genes and cytokine signal transduction.[6]
- Underlying multiple cytokine/inflammatory pathways in WM compared to IgM-MGUS was upregulation of miRs-146a, 150, 194, all targets of *STAT1*. Of relevance, miR-146a has been previously shown to be an inhibitor of inflammatory cytokines, including IL-6.
- A significant alteration of the FGF/FGFR axis which plays a role in the regulation of the TME structure was additionally observed. Here, FGF7, FGF13 and FGFR3 were downregulated, predicted to be targeted by miR-155, miR-16 and miR-194, all upregulated.

DISCUSSION AND CONCLUSION

- There were significant differences in miRNA expression between IgM-gammopathies-normal controls and WM-MGUS, with miRNA-based implication of multiple inflammatory and cytokine signalling pathways.
- In IgM-gammopathies, a miRNA-based activation of multiple cytokines which perform a known role in regulating B-cell maturation and the pathogenesis of B-cell malignancies were observed as compared to controls.
- In comparing WM to MGUS – a downregulation of multiple cytokine signaling pathways involved in inflammation and the tumor microenvironment, along with targeting of the STAT family, indicates a possible miRNA-based negative feedback loop to down-modulate inflammatory cytokine signaling in WM.
- Future investigation of miRNAs in regulation of these pathways may find novel therapeutic targets in the IgM-gammopathies disease spectrum.

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There are no disclosures or conflicts of interest for this work. Contact: **chohan.karan@mayo.edu**