Deficiency of immune regulators IRGM, TIFAB, and miR-146 in hematologic cancers

Caroline M. Putnam, Meredith B. A. Kesler, Lahari Kondeti, and **Melinda E. Varney, Ph.D.**

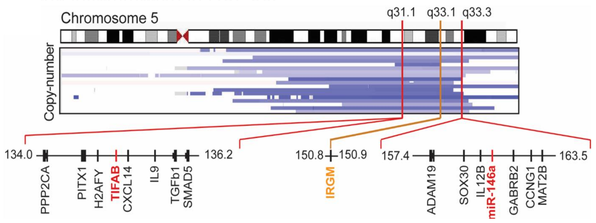
MARSHALL UNIVERSITY SCHOOL OF PHARMACY

Abstract

Our objective was to obtain a more comprehensive understanding of how loss of IRGM, TIFAB, and miR-146a contribute to del5q myelodysplastic syndromes (MDS). Del5q MDS arise from the deletion of a region of the short arm of human chromosome 5q. Three genes, Tifab, miR-146a, and Irgm, span a region of chromosome 5 that is commonly deleted. Each gene functions in innate immune response. Loss of TIFAB and miR-146a results in overactive TRAF6-mediated immune signaling in hematopoietic stem and progenitor cells (HSPCs). Less is understood about how loss of IRGM may contribute to disease. Due to a compensatory increase in gene expression of Irgm1, the mouse homolog of human IRGM, in Tifab-/-;miR-146a-/- double knockout mice and recent studies indicating involvement of IRGM in the regulating innate immune signaling, we hypothesized that loss of IRGM contributes to disease in a way that complements loss of TIFAB and miR-146a, by increasing pro-inflammatory signaling through modulating TRAF6. To test our hypothesis, we evaluated the effect of Irgm1 loss on TRAF6 gene expression. Bone marrow was extracted from wild type and Irgm1-/- knockout mice exposed to lipopolysaccharide (LPS). RNA was extracted from the bone marrow, converted to cDNA, and qPCR was performed. Traf6 gene expression was normalized to Gapdh for each mouse. Results indicate that Traf6 mRNA is significantly upregulated in the bone marrow of Irgm1-/- knockout mice. This suggests that loss of IRGM may contribute to del5q MDS through mechanisms that lead to overactivation of Traf6-mediated innate immune signaling. This work provides rationale for further investigation of Irgm1's role in the regulation of TLR4 signaling in HSPCs.

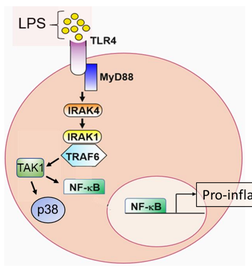
MDS:

- MDS are a group of malignant hematopoietic stem and progenitor (HSPC) cell disorders clinically defined by myeloid dysplasia, inefficient hematopoiesis, genetic instability, and predisposition to developing acute myeloid leukemia (AML).
- Del5q MDS have a deletion within chromosome (chr) 5q. Commonly deleted regions include chr 5q31.1 and 5q33.3.
- TIFAB, miR-146a, and IRGM1 are located in commonly deleted regions and function to negatively regulate immune signaling and inflammation in HSPCs.



IRGM1:

- IRGM regulates autophagy formation in response to pathogens.[1]
- Irgm1-deficient mice display impaired hemopoietic responses to infection.[1,2]
- In response to lipopolysaccharide (LPS), Irgm1 negatively regulates expression of MyD88-dependent genes through the regulation of p38 and NF-κB signaling in macrophages.[3]

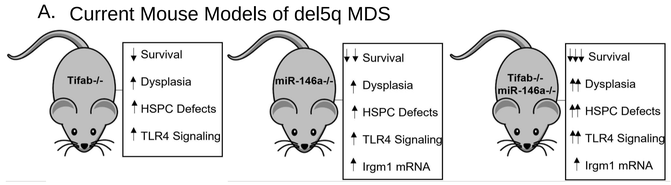


Toll-like receptor 4 (TLR4) signaling is initiated when pathogen associated molecular patterns (such as LPS) are recognized by TLR4. Subsequent MyD88 activation leads to TRAF6 mediated p38 and NFκB activation. NFκB translocation to the nucleus results in the transcription of pro-inflammatory genes.

Loss of TIFAB and miR-146a in mice induces IRGM1 expression

Current mouse models of del5q MDS experience decreased survival, blood and bone marrow dysplasia, hematopoietic defects, and increased TLR4 signaling in HSPCs.[4,5] RNAseq analysis of HSPCs in established MDS mouse models reveals that Irgm1 is upregulated miR-14a-/- and DKO mice. We anticipate that this is to compensate for lack of innate immune signaling regulation.

A. Current Mouse Models of del5q MDS



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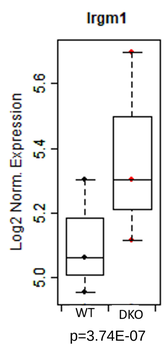
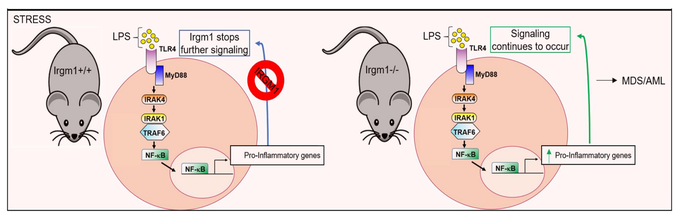


Figure 1: In addition to many MDS phenotypes exhibited in current mouse models of del 5q MDS, Tifab-/-;mir-146a-/- (DKO) mice exhibit an increase in Irgm1 expression when compared to wild type (WT) mice. (A) Phenotypes exhibited by current del5q MDS mouse models is summarized. (B) RNAseq analysis of HSPCs in established MDS mouse models reveals that Irgm1 is upregulated in miR-14a-/- (not shown) and DKO mice. The data for this analysis was obtained from Varney et al. *Leukemia* 2017. [5]

In humans, a deletion spanning Tifab and miR-146a would also result in deletion of Irgm1. Taken together with established functions of IRGM and IRGM1, this led to our investigation of IRGM deletion and its contribution to MDS pathogenesis.

Hypothesis

Loss of IRGM1 under stress-induced conditions contributes to overactive inflammation in hematopoietic stem and progenitor cells in mice.



Methods

Ex vivo studies: Bone marrow was extracted from wild type and Irgm1-deficient mice and cultured in StemSpanTM + 100ng/mL LPS for 8 hours. RNA was isolated and converted to cDNA. qPCR was performed to analyze TRAF6 and GAPDH gene expression.

In vivo studies: Wild type and Irgm1-deficient mice were injected with LPS (1mg/g body weight). 24 hours later, mice were euthanized. Spleens were weighed. Bone marrow was extracted. mRNA was isolated, converted to cDNA and subjected to qPCR analysis of many immune response genes.

In response to LPS, loss of IRGM1 leads to increased TRAF6 gene expression ex vivo

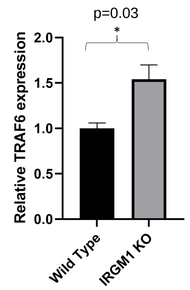
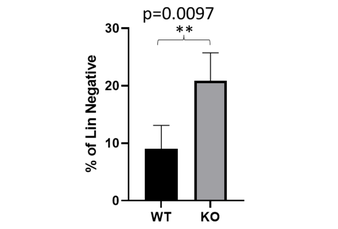


Figure 2: LPS treatment of Irgm1 knock out (KO) bone marrow increases TRAF6 mRNA expression. Bone marrow was extracted from wild type (WT) and IRGM1 KO mice. Red blood cells were lysed, and the remaining cells were cultured in StemSpan™ with 100ng/mL LPS for 8 hours. mRNA was isolated and subsequently converted to cDNA. qPCR was performed to analyze TRAF6 and GAPDH gene expression. An asterisk (*) indicates $p \leq 0.05$

In response to LPS, loss of IRGM1 leads to increased HSPCs and spleen size in mice

A.



Consistent with published findings:
King et al. 2011 *Blood*

B.

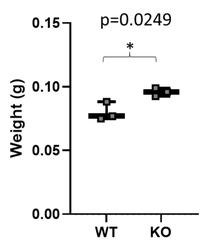


Figure 3: LPS treatment in Irgm1 knock out mice induces MDS-associated phenotypes in IRGM1 knockout (KO) mice when compared to wild type (WT) mice. WT and IRGM1 KO mice were injected with LPS (1mg/g body weight). After 24 hours, mice were euthanized. (A) HSPCs were assessed by flow cytometry. Lineage negative cells were analyzed for frequency of Sca-1 and c-Kit positive cells. (B) Spleen weight was recorded. An asterisk (*) indicates $p \leq 0.05$; two asterisks (**) indicate $p \leq 0.01$

In response to LPS, loss of IRGM1 leads to altered inflammatory signaling in vivo

A.

Upregulated Genes	Gene Function
Socs2	Catalyzes the transfer of electrons from Cytochrome C to molecular oxygen
Socs1	Catalyzes the transfer of electrons from cytochrome c to molecular oxygen and pump protons across the inner mitochondrial membrane
Ccr2	A receptor for monocyte chemoattractant protein 1
Cxcr3	G protein-coupled receptor that when bound, induces cellular responses that are involved in leukocytes traffic
Csf2	Cytokine that controls the production, differentiation, and function of granulocytes and macrophages
Cd19	Encodes for a member of the immunoglobulin gene superfamily, restricted to B cell lymphocytes
Stat6	A STAT family transcription factor that plays a role in exerting IL4 mediated biologic responses

B

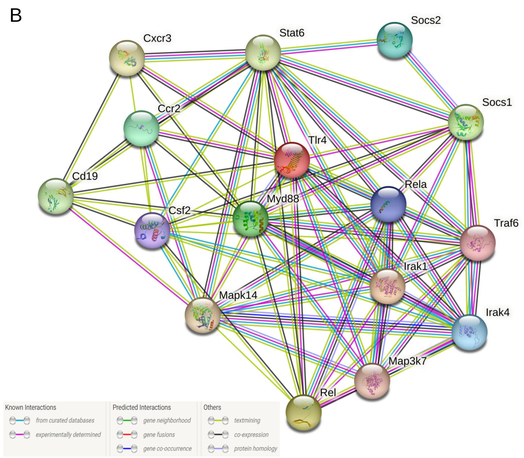


Figure 4: LPS treatment in Irgm1 knockout (KO) mice leads to upregulated gene expression of genes associated with TLR4 signaling. Wild type and Irgm1-deficient mice were injected with LPS (1mg/g body weight). After 24 hours, bone marrow was extracted. Red blood cells were lysed. RNA was isolated, converted to CDNA. qPCR was performed with Taqman Immune Response Assay. (A) Significantly upregulated genes are listed. (B) A String network was created in The STRING Database (https://string-db.org) by entering the gene/protein names from part A and members of the TLR4 signaling network.

Discussion

Our findings indicate that under stress induced by LPS, IRGM1 loss:

- Alters frequency of HSPCs in the bone marrow
- Induces splenomegaly, indicative of extramedullary hematopoiesis
- Alters TLR4 signaling in bone marrow

Acknowledgements

The authors would like to thank:

- Dr. Gregory Taylor (Duke University), Dr. Taylor provided the IRGM-/- mouse model to the Varney lab.
- The Marshall University animal facility staff for providing care and maintenance for the mice.
- Dr. Michael Hambuchen for assistance with i.p. injection of mice.

Sources of funding:

- Marshall University School of Pharmacy (Start-up funds),
- West Virginia IDeA Network for Biomedical Research Excellence (NIH Grant P20GM103434), and
- American Association of Colleges of Pharmacy (New Investigator Award).

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