SOHO ANNUAL MEETING 2024

SOX11 INHIBITS BCR SIGNALING THROUGH THE PAX5/CD19 AXIS FOR THERAPEUTIC TARGETING IN BTK AND BCL2 INHIBITOR RESISTANT MANTLE CELL LYMPHOMA

R. DUTTA[1], H. LEE[2], V. LESHCHENKO[1], R. SHUKLA[1], Y. LIU[2], S. RECI[1], L. GROSSMAN[1], J. JIN[3], M. WANG[2] , S. PAREKH[1]

[1]Hematology and Medical Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, The Precision Immunology Institute at Mount Sinai, New York, NY; [2]Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX; [3]Mount Sinai Center for Therapeutics Discovery, Department of Pharmacological Sciences, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY

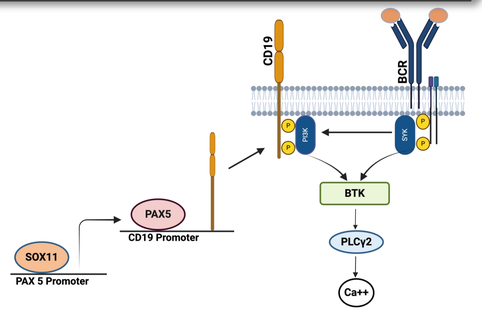
Mount Sinai

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

INTRODUCTION

- Mantle cell lymphoma (MCL) is an incurable lymphoid neoplasm, comprising about 7% of all non-Hodgkin lymphoma (NHL).
- The transcription factor SOX11 is overexpressed in the majority of MCL patients and associated with poor prognosis. In MCL, naïve B cells experience continuous proliferation because of the dysregulation of the B-cell receptor (BCR) pathway.
- Our previously published data (Kuo et. al., Blood 2018) identified that SOX11 aberrantly upregulated BCR signaling in transgenic mouse models. The current study describes the exact mechanism by which SOX11 regulates the BCR pathway using a combination of functional and pharmacological approaches.

HYPOTHESIS



METHOD

- SOX11 was depleted in human MCL cell lines Z-138 and JeKo-1 using shRNA and CRISPR.
- MAVER-1 and JeKo-1 stable cell lines were engineered for the ectopic expression of 3XFlag-tagged SOX11.
- PAX5 expression was assessed in peripheral blood mononuclear cells (PBMCs) from SOX11-Tg mice utilizing spectral flow cytometry.
- The impact of SOX11 DNA binding inhibitor (Jatiani et. al., 2021) and PROTAC was examined in MCL resistant cell lines (Zhao et., 2017; Huang et al., 2022) and animal tumor xenograft experiments.

RESULTS

I. SOX11 regulates CD19 expression and BCR signaling in MCL through PAX5

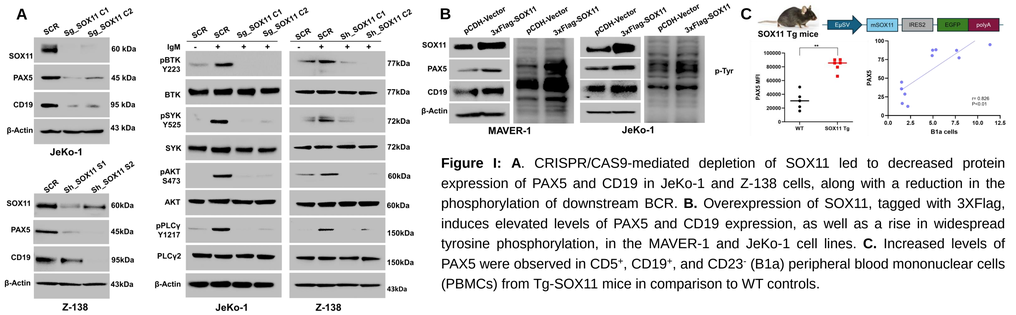


Figure I: A. CRISPR/CAS9-mediated depletion of SOX11 led to decreased protein expression of PAX5 and CD19 in JeKo-1 and Z-138 cells, along with a reduction in the phosphorylation of downstream BCR. **B.** Overexpression of SOX11, tagged with 3XFlag, induces elevated levels of PAX5 and CD19 expression, as well as a rise in widespread tyrosine phosphorylation, in the MAVER-1 and JeKo-1 cell lines. **C.** Increased levels of PAX5 were observed in $CD5^+$, $CD19^+$, and $CD23^-$ (B1a) peripheral blood mononuclear cells (PBMCs) from Tg-SOX11 mice in comparison to WT controls.

II. ChIP-Seq analysis demonstrates PAX5 is a direct target of SOX11

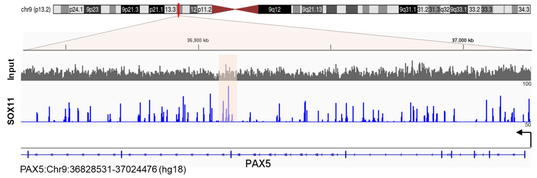


Figure II: ChIP-Seq in Granta-519 cells demonstrates enrichment of SOX11 at the PAX5 promoter site, suggesting direct binding. Integrative Genomics Viewer tracks showing the distribution of PAX5. Reads were aligned to the Homo sapiens genome Hg18 (Kuo et. al., Oncogene 2015).

III. SOX11i suppresses SOX11-mediated BCR signaling and halts MCL tumor cell proliferation in vivo

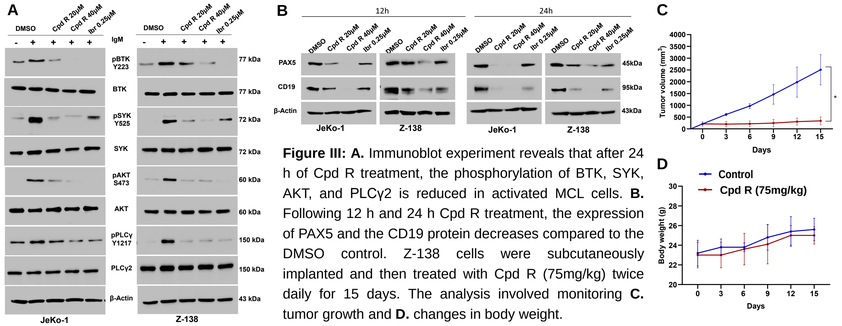


Figure III: A. Immunoblot experiment reveals that after 24 h of Cpd R treatment, the phosphorylation of BTK, SYK, AKT, and PLCγ2 is reduced in activated MCL cells. **B.** Following 12 h and 24 h Cpd R treatment, the expression of PAX5 and the CD19 protein decreases compared to the DMSO control. Z-138 cells were subcutaneously implanted and then treated with Cpd R (75mg/kg) twice daily for 15 days. The analysis involved monitoring **C.** tumor growth and **D.** changes in body weight.

IV. SOX11 inhibitor promotes apoptotic cell death in resistant MCL cells.

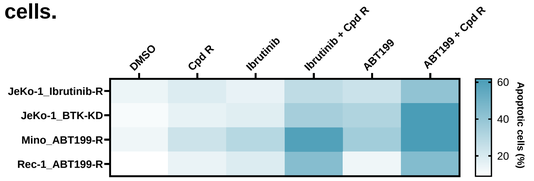


Figure IV: SOX11 inhibitor, alone or combined with ibrutinib and venetoclax, enhances apoptotic cell death in resistant MCL cells. Apoptosis was measured using an Annexin V/PI assay in ibrutinib-resistant cell lines (JeKo-1-Ibrutinib-R and JeKo-1-BTK-KD) and venetoclax-resistant cell lines (MINO-ABT-199-R and Rec-ABT-199-R) after 24 h of co-treatment with compound R (20μM) with ibrutinib (10 μM) or ABT199 (500 nM).

V. PROTAC capable of degrading SOX11 in MCL cells

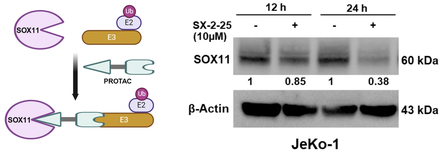


Figure V: Western blotting analysis of SOX11 protein expression in MCL cell line JeKo-1 treated with SOX11 degraders. JeKo-1 cells were treated with 10μM for 12 h and 24 h. SOX11 protein expression quantification was determined by densitometry using ImageJ. The numbers represent the SOX11-to-actin ratio.

CONCLUSIONS

- Our research delineates PAX5 as the direct target and PAX5/CD19 axis as the downstream pathway by which SOX11 regulates B-cell receptor (BCR) signaling in MCL.
- Treatment of MCL cell lines with small-molecule SOX11 inhibitors or degraders phenocopied the effects observed with genetic knockdown. Additionally, we confirmed the cytotoxic effects of SOX11 inhibitors in MCL-resistant cell lines and xenograft models.
- In summary, our research offers a novel understanding of the molecular pathways through which SOX11 influences MCL development. Pharmacological inhibitors of SOX11 show potential as a beneficial treatment approach for MCL patients, as they operate upstream of BTK.

REFERENCES

1. Kuo P. Y et al. High-resolution chromatin immunoprecipitation (ChIP) sequencing reveals novel binding targets and prognostic role for SOX11 in mantle cell lymphoma. Oncogene. 2015; 34(10), 1231–1240.
2. Kuo P. Y et al. SOX11 augments BCR signaling to drive MCL-like tumor development. Blood. 2018;131(20), 2247–2255.
3. Jatiani S. S et al. SOX11 Inhibitors Are Cytotoxic in Mantle Cell Lymphoma. Clinical Cancer Research. 2021; 27(16), 4652–4663.
4. Zhao X et al. Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma. Nat Communications 2017; 18 (8), 14920.
5. Huang S et al. PIK-75 overcomes venetoclax resistance via blocking PI3K-AKT signaling and MCL-1 expression in mantle cell lymphoma. Am J Cancer Res. 2022; 12(3), 1102-1115.

ACKNOWLEDGEMENTS

The study was sponsored by NIH R01CA252222 and the Icahn School of Medicine at Mount Sinai grant “Structure Guided SOX11 PROTAC Development” is gratefully acknowledged.

CONTACT INFORMATION

Rudra Prasad Dutta

Parekh Lab, Icahn School of Medicine at Mount Sinai, NY

rudra.dutta@mssm.edu; samir.parekh@mssm.edu

https://www.parekhlab.com/