Targeting of Myc -| miR30a -| PTP4A1→WNT axis in Double Hit Lymphomas

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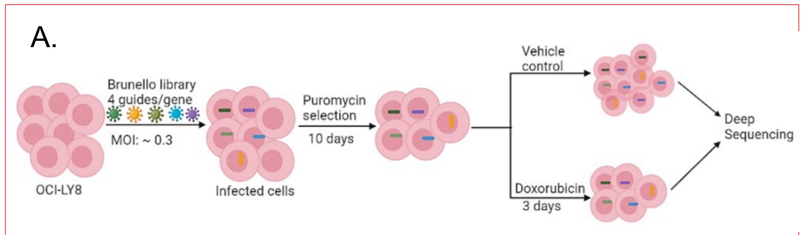
Abstract

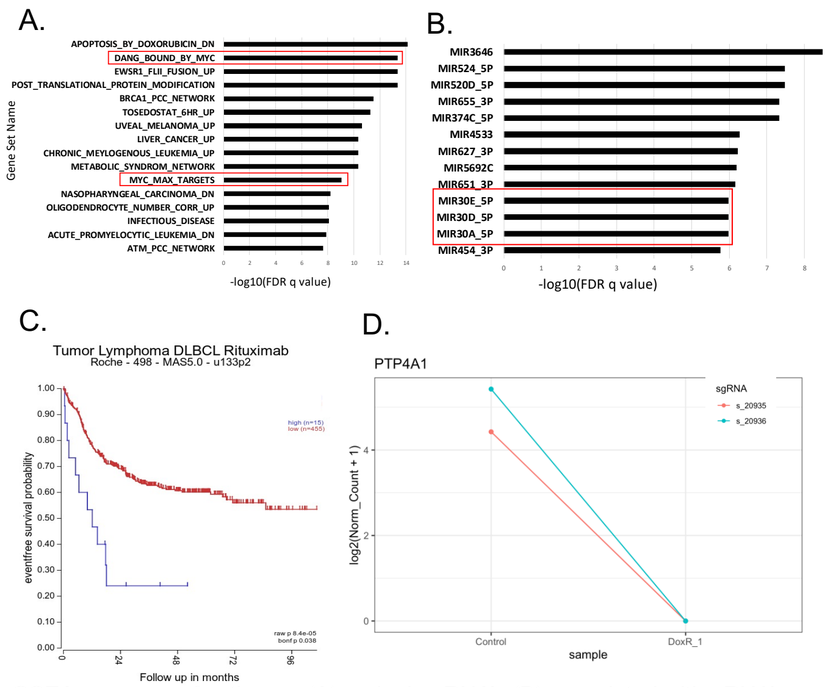
Diffuse large B-cell lymphomas (DLBCL) have frequently rearranged Myc along with the anti-apoptotic gene BCL2, and these double-hit lymphomas (DHL) are known to be largely refractory to standard chemotherapy. This underscores the need for new treatment strategies targeting or bypassing BCL2. While small-molecule inhibitors of BCL2 (venetoclax, etc) are emerging as standards of care, acquired resistance continues to be a barrier to successful treatment. To simultaneously identify resistance mechanisms and additional therapeutic targets in DHL, we have conducted a genome-wide CRISPR/Cas9 dropout screen with doxorubicin and vehicle treated cells. 900 significantly depleted guides corresponding to putative resistance genes were selected for Gene Set Enrichment Analysis. Among curated datasets, we observed enrichment for direct genomic targets of Myc, targets of miR-30a microRNA, as well as Wnt pathway components. Remarkably, the PTP4A1 gene encoding a protein tyrosine phosphatase appeared in all 3 lists, suggesting that it could be the key druggable node controlling therapeutic responses. Using Wnt pathway inhibitor ICG-001, we discovered that Wnt signaling is necessary for DHL survival in vitro potentially via MYC -| miR-30a -| PTP4A1 axis.

Background



(A) Schematic illustrates the workflow of genome-wide CRISPR/Cas9 knockout library screen with Doxorubicin in OCI-Ly8 cells. (B) Score graph showing top genes corresponding to significantly depleted guides in CRISPR/Cas9 drug screen.

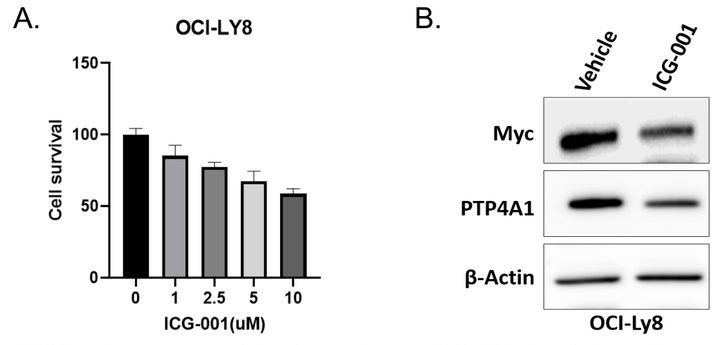
Identification of Wnt, Myc, miR-30a and PTP4A1 axis in Doxorubicin treated cells



GSEA summary of pathways (A) and microRNA's (B) associated with gRNA's depleted in 1nM doxorubicin treated OCI-Ly8 cells. (C) Kaplan Meier curve showing association of PTP4A1 with survival of DLBCL patients. (D) The sgRNAs targeting PTP4A1 were consistently depleted in Doxorubicin treated cells.

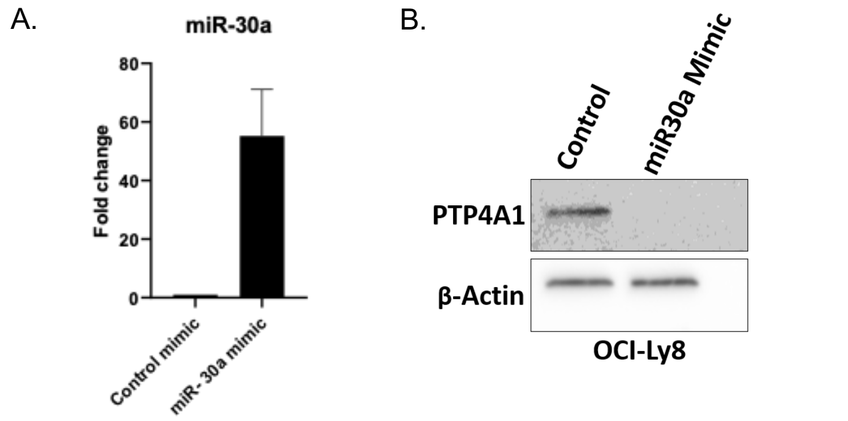
Hypothesis: In DHL and other aggressive lymphomas, MYC -| miR-30a -| PTP4A1→Wnt form an oncogenic axis driving cell proliferation and drug resistance.

Wnt pathway inhibition by ICG-001 decreases cell survival



(A) WST-1 Assay with Cells treated with ICG-001 for 24hrs. (B) Immunoblot showing Myc and PTP4A1 levels in cells treated with vehicle and 10 μM ICG-001 for 24 hrs.

miR-30a mimic decreases PTP4A1 expression



(A) Quantitation of miR-30a expression normalized to RNU6B (log2-transformed ratio) by qRT-PCR.
(B) Immunoblot showing PTP4A1 levels in control and miR30a mimic transfected cells. β-Actin serves as loading control.

Future Directions

- Validate the top hits from the screen.
- Establishing PTP4A1 as miR-30a target.
- Establishing Myc-miR30a-PTP4A1 axis role in drug resistance of DLBCL cells.
- Using Wnt inhibitor for chemosensitization of DLBCL cells.
- Using PTP4A1 inhibitor for chemosensitization of DLBCL cells
- Validate effects of Wnt and PTP4A1 inhibitors in PDX models.

References

1. McMahon SB. MYC and the control of apoptosis. Cold Spring Harb Perspect Med. 2014;4(7):a014407.
2. Friedberg JW. How I treat double-hit lymphoma. Blood. 2017;130(5):590-596.
3. Bose P, Gandhi V, and Konopleva M. Pathways and mechanisms of venetoclax resistance. Leukemia Lymphoma. 2017;58(9):2026-2039.
4. Sanson KR, Hanna RE, Hegde M, Donovan KF, Strand C, Sullender ME, et al. Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nat Commun. 2018;9(1):5416.
5. Graessmann M, Berg B, Fuchs B, Klein A, and Graessmann A. Chemotherapy resistance of mouse WAP-SVT/t breast cancer cells is mediated by osteopontin, inhibiting apoptosis downstream of caspase-3. Oncogene. 2007;26(20):2840-50.
6. Zeller KI, Jegga AG, Aronow B, O'Donnell KA, and Dang CV. An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. Genome Biol. 2003;4:R69.1-R69.10.
7. Wei M, Korotkov KV, and Blackburn JS. Targeting phosphatases of regenerating liver (PRLs) in cancer. Pharmacol Therapeut. 2018;190:128-138.
8. Swier L, Dzikiewicz-Krawczyk A, Winkle M, van den Berg A, and Kluiver J. Intricate crosstalk between MYC and non-coding RNAs regulates hallmarks of cancer. Mol Oncol. 2019;13(1):26-45.

Acknowledgements

Thank you to Prof. Andrei Thomas-Tikhonenko for his mentoring and sponsorship, Priyanka Sehgal PhD, for her mentoring and assistance, Colleen Harrington PhD and Ruchi Patel for their work on the CRISPR/Cas9, and all the members of the Thomas-Tikhonenko Lab for their support.