Summer 8-10-2023

Investigating the Role of TP53 in Peripheral T-Cell Lymphoma-GATA3 Subtype

Zaina W. Nasser
University of Nebraska - Lincoln, University of Nebraska Medical Center

Dylan T. Jochum
University of Nebraska Medical Center

Waseem G. Lone
University of Nebraska Medical Center

Alyssa C. Bouska
University of Nebraska Medical Center

Sunandini Sharma
University of Nebraska Medical Center

See next page for additional authors

Author

Zaina W. Nasser, Dylan T. Jochum, Waseem G. Lone, Alyssa C. Bouska, Sunandini Sharma, and Javeed Iqbal

Investigating the Role of TP53 in Peripheral T-Cell Lymphoma-GATA3 Subtype

Zaina W. Nasser[1,2], Dylan T. Jochum[1], Waseem G. Lone[1], Alyssa C. Bouska[1], Sunandini Sharma[1], Javeed Iqbal[1]

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE[1], University of Nebraska-Lincoln, Lincoln, NE[2]

UNMC UNIVERSITY OF NEBRASKA MEDICAL CENTER

Summer **U**ndergraduate **R**esearch **P**rogram 2023

Introduction

Non-Hodgkin Lymphoma (NHL) accounts for 4.1% of all cancers in the United States. Peripheral T-Cell Lymphoma (PTCL) consists of ~10-15% of all NHL in the Western world[1]. 30-50% of these PTCLs are not classifiable/diagnosed and are instead designated as PTCL-Not Otherwise Specified (PTCL-NOS). Based on the molecular signatures below, the two major molecular subgroups within PTCL-NOS are PTCL-TBX21 and PTCL-GATA3, determined by their distinct T-helper (T_H) transcriptional programs. GATA3 and TBX21 are the master transcriptional regulators of T_H2- and T_H1-cell differentiation, respectively[2]. The overall survival analysis of PTCL-NOS cases illustrates the clinical outcome of PTCL-GATA3 cases are significantly lower than PTCL-TBX21 cases over a broad timeframe[3]. Thus, the need for understanding the underlying mechanism and finding therapeutic targets is at the utmost importance.

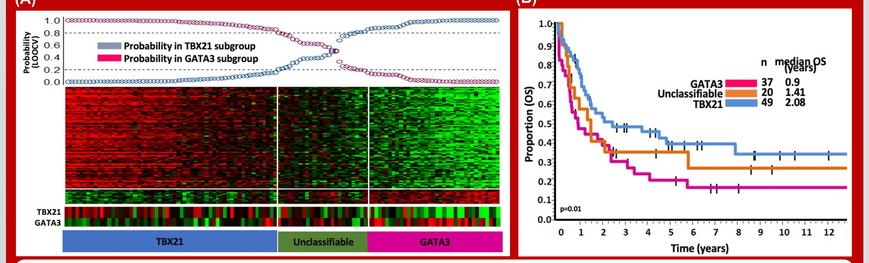


Figure 1: PTCL-NOS has two major molecular subgroups. (A) Molecular signatures and gene expression profiles in T-helper transcriptional programs in PTCL-NOS identified two major subtypes, PTCL-GATA3 and PTCL-TBX21, based on Bayesian prediction **(B)** Kaplan-Meier curves of these subtypes based on these classifications, showing a significant difference (p=0.01).

Background

As seen in the mutation plot below, TP53 mutations and/or TP53 loss deletions are frequent in PTCL-GATA3 cases, compared to PTCL-TBX21. TP53 is a protein that is essential in cycle regulation but also acts as a tumor suppressor. It stops cells from dividing if they have mutated or damaged DNA[4]. Due to the high mutation rates observed in this subtype, we believe TP53 could play a major role in this mechanism. Therefore, it was important to focus on the TP53-GATA3 interaction at the genomic level. Prior studies using chromatin immunoprecipitation (ChIP)-qPCR on the intron 3 full GATA3 region suggested there was more TP53 binding in this intron region compared to other regions. Therefore, we designed a research strategy to determine the specific binding regions of TP53-GATA3 interaction and the function of the TP53 binding.

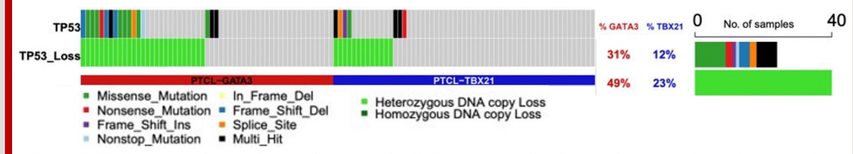


Figure 2: TP53 is frequently genetically altered in PTCL-GATA3 compared to other subtypes. Mutation plot displaying the frequencies of TP53 mutation and copy number loss in PTCL-GATA3 cases compared to PTCL-TBX21

Results

Hypothesis: TP53 is involved in transcriptional regulation of GATA3

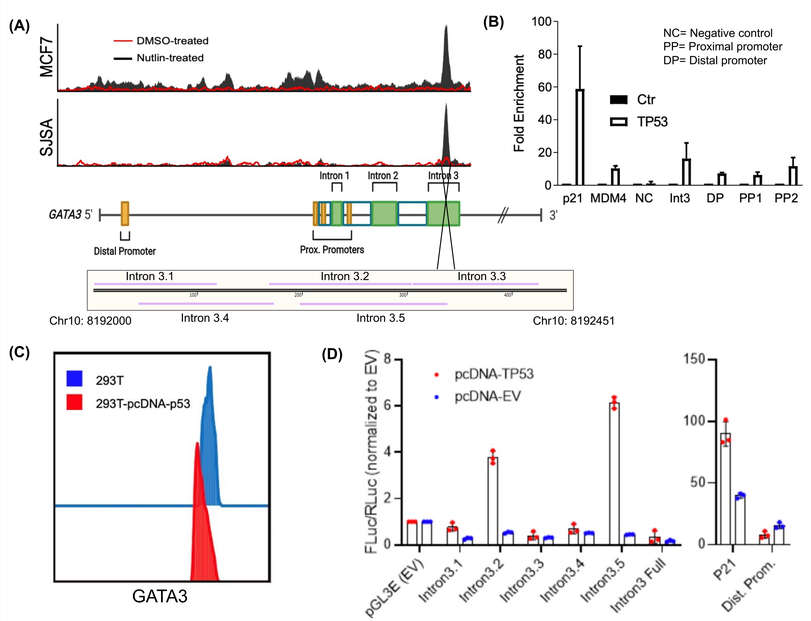
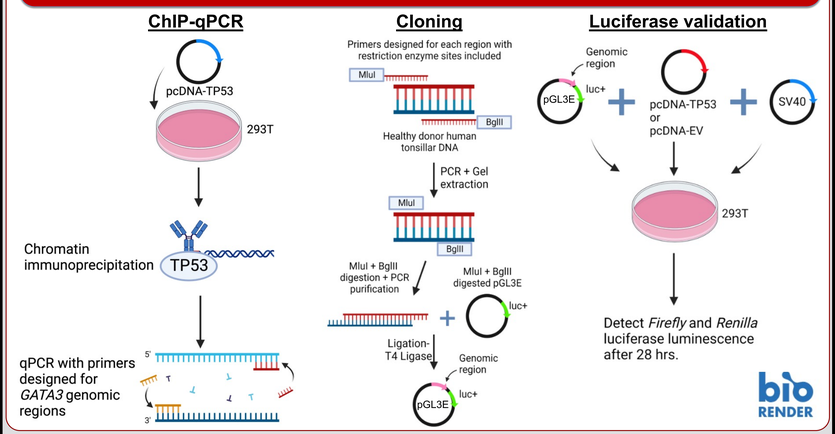


Figure 3: TP53 mechanistically plays a role in GATA3 regulation. (A). CHIP-seq data illustrates MCF7 and SJSA cell lines show increased binding within GATA3, particularly increased binding within third intron region (amplified regions for these current experiments are highlighted). **(B)** Fold enrichment in CHIP-qPCR validates the significant amount of p53 binding along GATA3 regulatory regions illustrated in the ChIP-seq. **(C)** Flow cytometry data shows as p53 increases, GATA3 decreases. **(D)** GATA3 intron 3 regions, GATA3 distal promoter, and controls (empty vector and p21) were cloned into pGL3-E vectors and transfected into 293T cells. Luciferase activity for every vector combination was determined by dividing *Firefly* luminescence by *Renilla* luminescence, with or without pcDNA-TP53 (TP53 expression vector).

Methods



Conclusion

- CHIP-qPCR, in conjunction with the luciferase assay, shows high binding, but low luciferase activity in the intron 3 region, specifically at the loci labeled as intron 3.1, 3.3, and 3.4.
- This experiment indicates that TP53 binding leads to GATA3 inhibition, which suggests that TP53 plays a direct role in the negative regulation of GATA3, and thus T_H2-cell differentiation, beyond its canonical role in cell cycle regulation. P21 (CDKN1A) was used as a positive control to validate the activity of the TP53 expression, where we observed an increase in luciferase expression when TP53 was increased, as expected.
- From the full intron 3 region of GATA3, the region has been narrowed down to focus on the intron 3.1, 3.3, and 3.4 regions to determine the exact location of TP53-GATA3 interaction and could be used to identify other potential proteins involved in the interaction, for the future experiments.

Future Directions

- **Repeat the assay with mutated sites (via site-directed mutagenesis) where low luciferase expression was observed**
- **Validate in murine and human PTCL models**
- **Pull down DNA-protein complexes at these loci and identify if other proteins involved**
- **Cut-and-run sequencing to identify other TP53 targets that could be involved in indirect GATA3 regulation**

References

[1] National Cancer Institute. 2018. Non-Hodgkin Lymphoma - Cancer Stat Facts. *SEER*.
[2] Amador, et al. Reproducing the molecular subclassification of peripheral T-cell lymphoma–NOS by immunohistochemistry. *Blood*, 2019.
[3] Iqbal, et al. Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. *Blood*, 2014.
[4] Medline Plus. 2020 Aug. TP53 gene: MedlinePlus Genetics. *medlineplus*.

Acknowledgments

Thank you to Dr. Javeed Iqbal and the entire Iqbal lab, with special recognition to Dylan, for his mentoring and guidance throughout this project. This work would not have been possible without UNMC's Summer Undergraduate Research Program (SURP).