Deciphering the multifaceted roles of a novel ultraconserved noncoding RNA, overexpressed in Chronic Lymphocytic Leukemia

Paola Rausseo, Swati Mohapatra, Erik Knutsen, George A. Calin

Department of Translational Molecular Pathology, MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction

Chronic Lymphocytic Leukemia (CLL) is a malignancy of CD5+ B cells that primarily occurs in the blood and bone marrow. It's one of the most common blood cancers in Western countries and about 10% of patients diagnosed with CLL develop into Richter Syndrome (RS), an extremely aggressive transformation into lymphoma.

Ultra-conserved regions (UCRs) are genomic regions that are perfectly (100%) conserved in between humans, mice and rats. Some of the UCRs get transcribed into long noncoding RNAs often called as Transcribed Ultraconserved regions. Perfect conservation of T-UCRs makes them interesting and aberrations in such regions has been correlated to cancer susceptibility. TRUC-16 is found to be overexpressed in aggressive CLL and Richter patients. Around 4% of patients exhibit G324A mutation in this region. Direct interaction of TRUC-16 to p16INK4A leads to lower levels of p16INK4A and overexpressed conditions of TRUC-16. P16INK4A, a tumor suppressor, is found to be silenced in 30% of Richter Transformation cases.

Hypothesis:

We hypothesize that forced overexpression of this ncRNA along with G324A mutation in B cells leads to aggressive transformation into RS-like phenotype in C57BL6 mice. Such aggressive transformation potentially leads to migration of naïve B cells from peripheral system into spleen and lymph nodes that eventually drives the transformation into lymphoma.

Methods

In order to investigate the disease, we phenotyped CRISPR-Knockin mice, generated in Dr. Calin's lab. These mice have B-Cell specific overexpressed TRUC-16 given that uc.206 is under control of Vh promoter and IgH-Eμ enhancer which target expression to B-cell compartment..

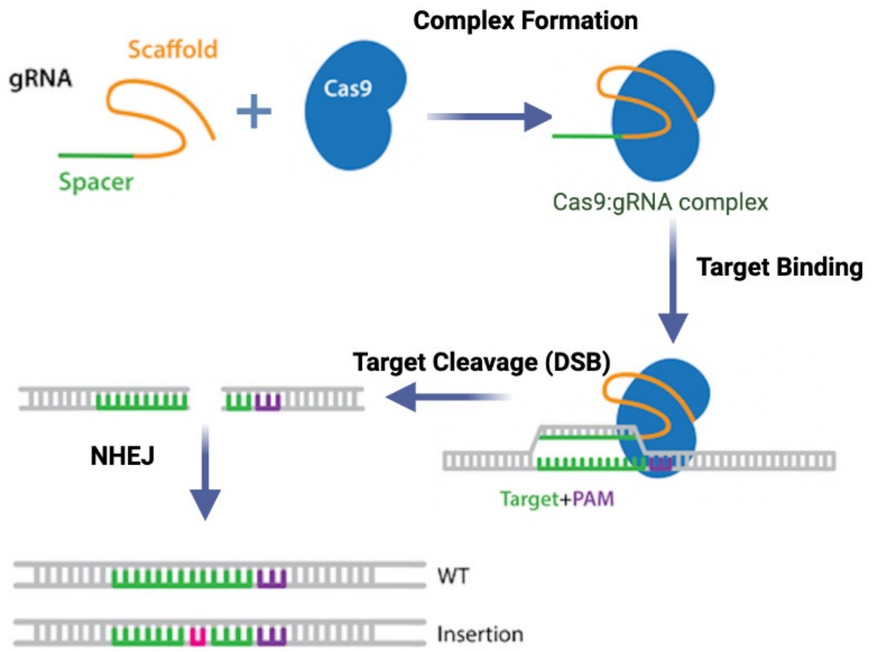

Figure 1: *CRISPR Cas9 Knock-In strategy. Cas9 with donor plasmids were injected into zygotes of mice. Backcrossing was performed to obtain pure breeds which were subjected to further genotyping.*

Transgenic mice cohorts (TgMut) were subjected to genotypic screening. Genotyping is a very important step in our experiments. This lets us tell apart the mice from Heterozygous, homozygous insert and wildtype littermates. Genotyping is done by snipping a small part of the mouse's ear/tail which is then lysed in the lab until its homogeneous. Afterwards, a PCR is performed with the go taq green protocol and then loaded into a 2% gel to assess the different base pairs in each well.

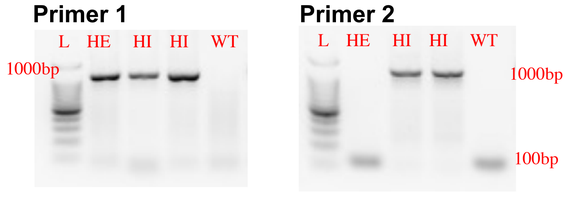

Figure 2: *Genotyping of B-cell specific overexpress TRUC-16 mice subjected to two sets of nested primers. L: Ladder; HE: Heterozygous; HI: Homozygous Insert; WT: Wildtype.*

Furthermore, we performed flow cytometry to assess lymphocytes in the peripheral blood. Flow cytometry analysis was performed using two panels comprising of CLL markers (CD5, B220, CD19, IgK and Igλ) and Richter panel including CD23, CD20, and Ki-67. Retro-orbital bleedings were performed on mice to collect peripheral blood that was further subjected to surface and intracellular staining. Data was acquired using BD LSR Fortessa and further analyzed using FlowJo software.

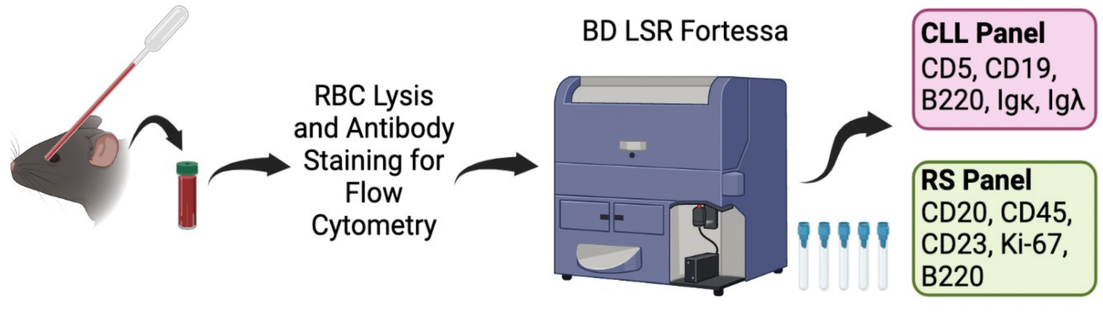

Figure 3*. Process of Flow Cytometry. Blood collection from mice through retro-orbital bleeding followed by immunophenotyping through multiparameter flow cytometry analysis.*

Results

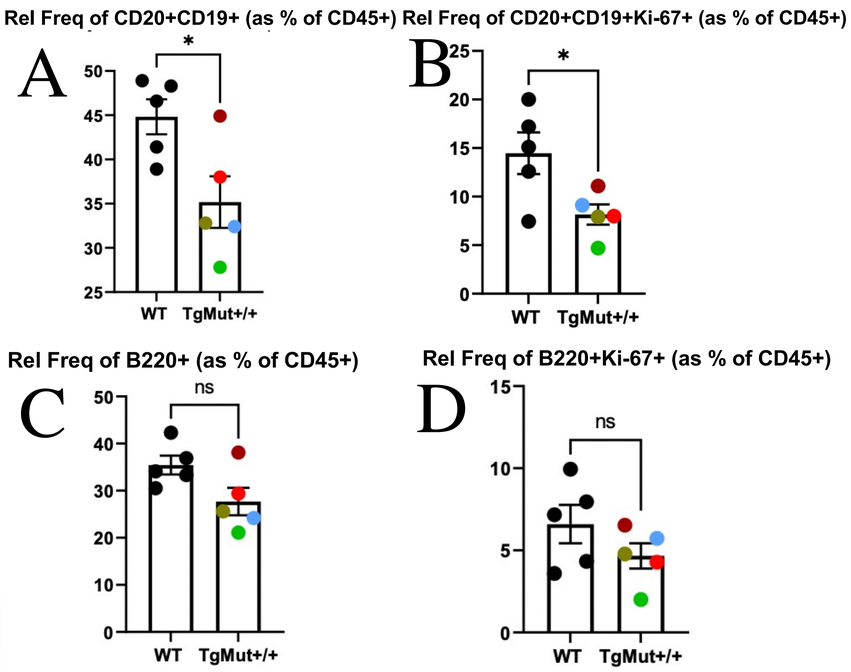

Figure 4. *A depicts lower CD20+ CD19+ immature B cells in the peripheral blood of TgMut++ mice compared to wildtype littermates. B shows lower proliferating immature B cells in TgMut++ mice compared to wildtype littermates. C and D shows lower population of immature B cells in the peripheral blood of TgMut++ mice compared to wildtype littermates validating the findings in A and B. TgMut depicts mice having overexpression of TRUC-16 along with G324A mutation*

Conclusion

Our results depict lower population of immature B cells in the peripheral blood of TgMut++ mice compared to the wildtype littermates. Potentially, these immature B cells are migrating to the spleen and lymph node during the initiation of transformation that needs to further investigated. Preliminary data from the lab show that high grade B cell lymphoma in adult TgMut++ mice (data not shown).

Future Directions

Flow cytometry analysis using CLL and RS Panel on single suspension cells from spleen and lymph nodes. Further, to characterize the B cell subtype, we would design a new panel utilizing CD80, CD27, CD138 and other B cell markers to better understand the B cell population in the immune compartment of these TRUC-16 overexpressing mice.

Acknowledgements

I would like to thank Swati, Dr. Calin and the Calin lab for mentorship and guidance. SM is supported by the CPRIT Research Training Grant (RP210028). GAC receives funding from the CLL Moonshot program at MDA.

References

Bejerano G, Pheasant M, Makunin I, et al. Ultraconserved elements in the human genome. Science. 2004;304(5675):1321-1325

Calin, G. A., Liu, C. gong, Ferracin, M., Hyslop, T., Spizzo, R., Sevignani, C., Fabbri, M., Cimmino, A., Lee, E. J., Wojcik, S. E., Shimizu, M., Tili, E., Rossi, S., Taccioli, C., Pichiorri, F., Liu, X., Zupo, S., Herlea, V., Gramantieri, L., … Croce, C. M. (2007).
Ultraconserved Regions Encoding ncRNAs Are Altered in Human Leukemias and Carcinomas. Cancer Cell, 12(3), 215–229.

Wojcik, S. E., Rossi, S., Shimizu, M., Nicoloso, M. S., Cimmino, A., Alder, H., Herlea, V., Rassenti, L. Z., Rai, K. R., Kipps, T. J., Keating, M. J., Croce, C. M., & Calin, G. A. (2010). Non-codingRNA sequence variations in human chronic lymphocytic leukemia and colorectal cancer. Carcinogenesis, 31(2), 208–215. https://doi.org/10.1093/carcin/bgp20