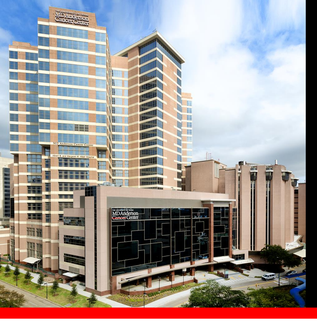

The involvement of miR-21 in the molecular pathogenesis of Richter transformation

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Introduction

- miRNAs are non-coding RNA molecules that play roles in posttranscriptional gene regulation and in translation.
- Our preliminary data indicates that miR-21 is overexpressed in patients with Richter transformation (RT).
- Therefore, the investigating the role of miR-21 could be associated with the development of RT in patients with chronic lymphocytic leukemia (CLL).
- However, its role in RT remains unknown.

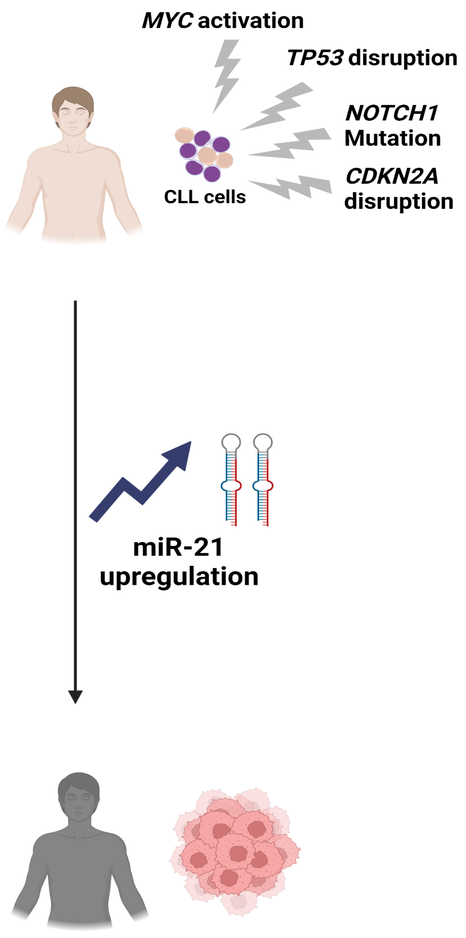

Figure 1. *miR-21 is significantly upregulated at the time of RT when compared to at CLL diagnosis.*

Hypothesis

In this study, we hypothesized that miR-21 overexpression is related to the molecular pathogenesis of RT

Research Aims

- Generate knock-out (KO) miR-21 stable CLL and RT cells using CRISPR/Cas9 technology.
- Determine effects of miR-21 on the cell cycle, apoptosis, and cell viability

Methods

- miR-21 KO cells were generated using CRISPR-Cas9 technology
- Genomic DNA extraction, PCR, and gel electrophoresis were conducted to genotype knockout cells.
- Sanger sequencing was conducted to sequence specific areas of knockout cells.
- RNA extraction, cDNA synthesis, and RT-qPCR were conducted to compare miRNA levels
- Cell viability was measured MTS assay.
- Flow cytometry was performed to determine the effects the miRNAs had on the cell cycle and apoptosis

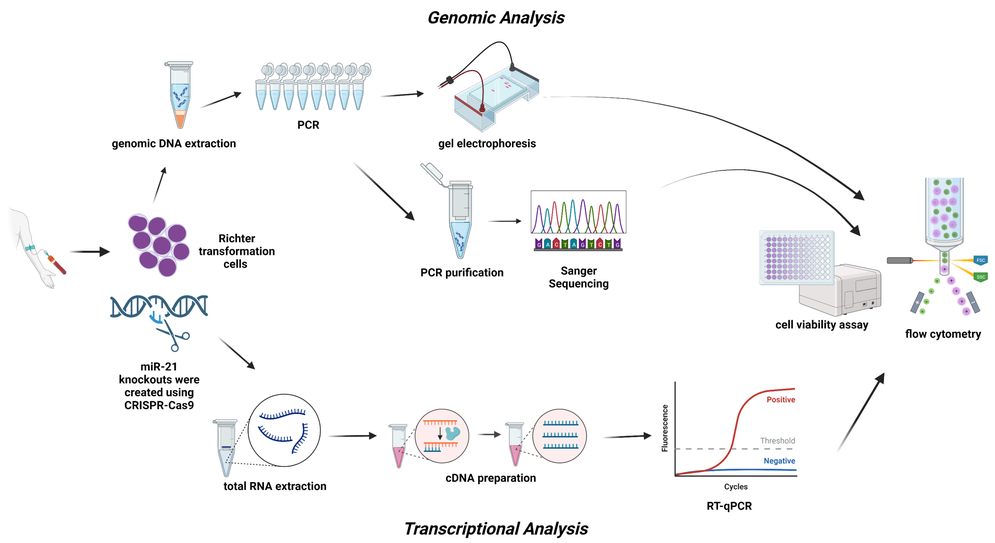

Figure 2. *Experimental workflow.*

Results

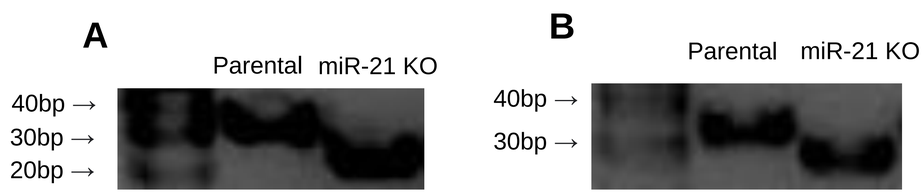

Figure 3. *Gel electrophoresis for parental and miR-21 knockout cells.*
(A) DNA was extracted from the MEC1 cell line
(B) DNA was extracted from the RT5 cell line

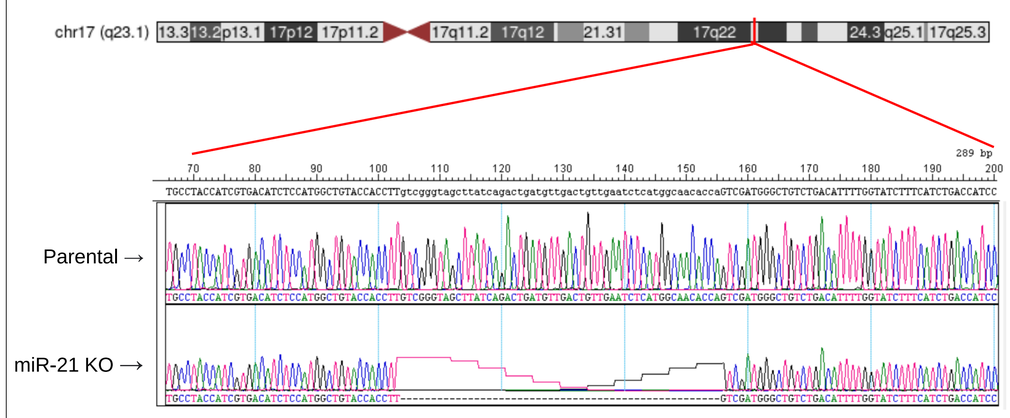

Figure 4. *Sanger sequencing for miR-21 KO cells.*

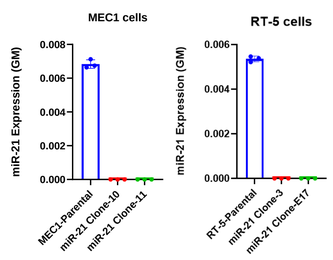

Figure 5. *miR-21 expression levels for KO cells.*

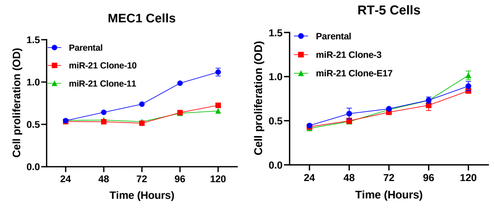

Figure 6. *miR-21 KO cells induce proliferation of cells.*

Results

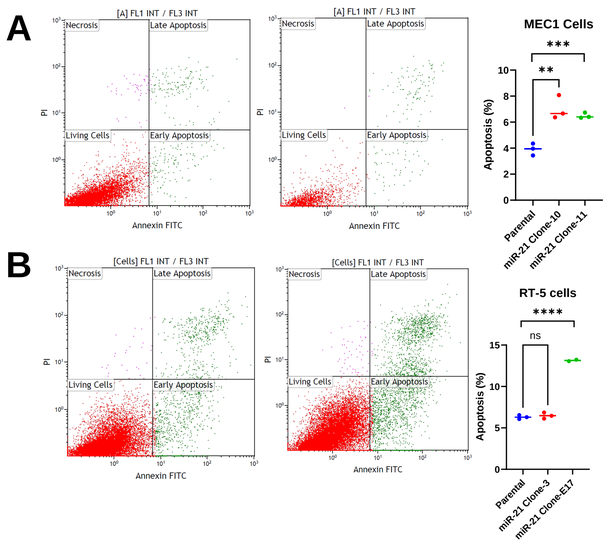

Figure 7. *miR-21 KO cells induce apoptosis.*
(A) Flow graphs for MEC1 cell line (parental on left)
(B) Flow graphs for RT5 cell line (parental on left)

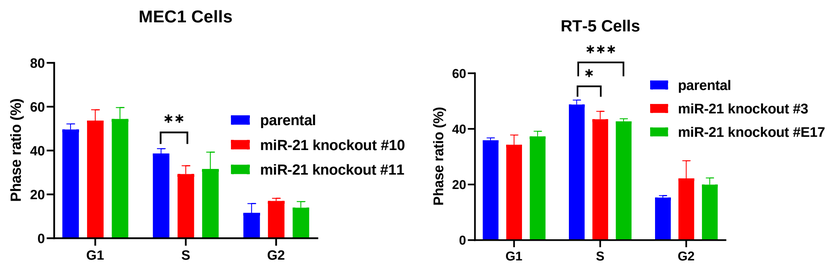

Figure 8. *miR-21 KO cells decrease cell proliferation and cell count in S phase.*

Conclusions

- We confirmed the oncogenic role of miR-21 in CLL and Richter transformation.
- Potential targets for miR-21 should be identified for future research
- The pre-Richter mouse model can be used with miR-21 knockout cells for future investigations.
- Exploring the function and possible implications of miR-21 during the transition from CLL to RT is a crucial area of investigation since miR-21 could potentially serve as therapeutic target.

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