The Genomic and Transcriptomic Landscape of Ultra-Conserved miR-142 in Hematological Malignancies

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Introduction

- MiRNAs are non-coding RNAs that regulate gene expression by targeting the 3'UTR of mRNAs with dysregulation often being linked to cancer
- Ultra-conserved among vertebrates (identical in rat, mouse, and human), miR-142 plays diverse physiological roles in the human body, controlling cellular functions such as apoptosis, proliferation, immune response, detoxification, and tumorigenesis[1-3]
- Our lab demonstrated that mutations are frequently concentrated in miR-142 and hematological malignancies, such as chronic lymphocytic leukemia (CLL)

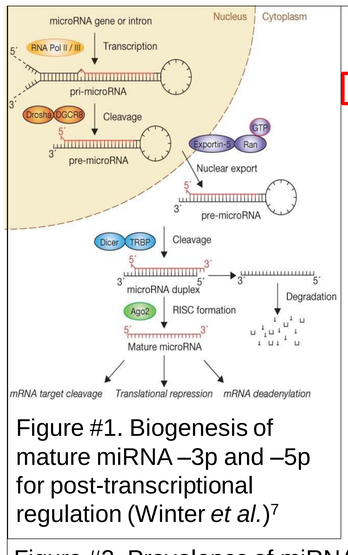

Figure #1. Biogenesis of mature miRNA –3p and –5p for post-transcriptional regulation (Winter *et al.*)[7]

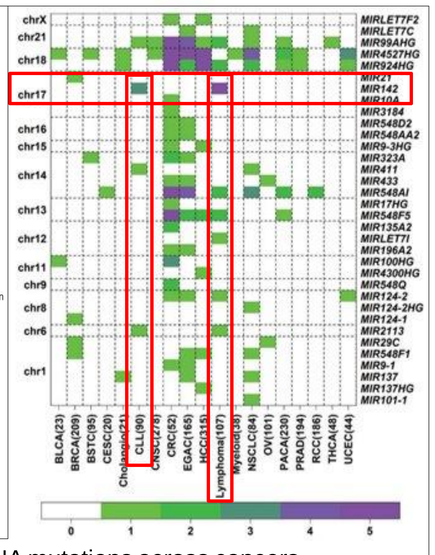

Figure #2. Prevalence of miRNA mutations across cancers.

- Hematological malignancies also showed the highest expression of mature miR-142 strands miR-142-3p and miR-142-5p

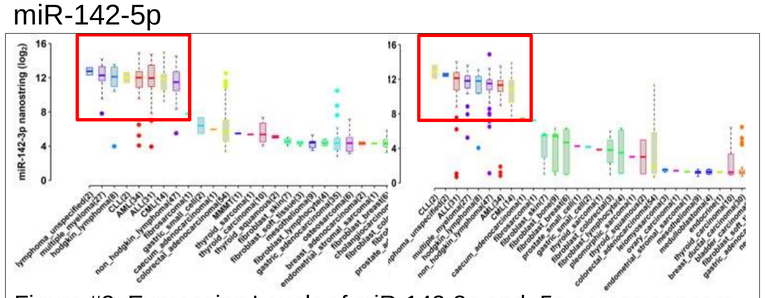

Figure #3. Expression Levels of miR-142-3p and -5p across cancers.

- However, there are still gaps of knowledge between specific miR-142 mutations and the effected mechanisms in chronic lymphocytic leukemia (CLL)

- We hypothesize that specific miR-142 mutations are inhibiting the processing of mature miR-142-3p and –5p forms in CLL

Method

- Targeted deep sequencing of ultra-conserved elements (UCEs) of 348 clinically well-annotated cancers was performed by MD Anderson and collaborating institutions
- Sanger sequencing was conducted in the UCE_5578 region containing miR-142 of 400 CLL cancer patient genomic samples (200 indolent; 200 aggressive)
- HEK293 cell lines were transfected with retroviral plasmids expressing miR-142 scrambled, wild-type, or mutated at the 6th nucleotide downstream
- RNA extraction, reverse transcription, and RT-qPCR were conducted to compare basal expression levels of pre-miR-142, miR-142-3p, and miR-142-5p

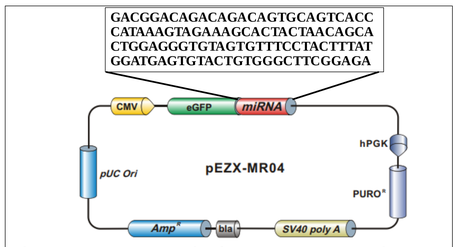

Figure #4. Retroviral UCE_5578–miR-142 Expressing Plasmid (GeneCopoeia)[5]

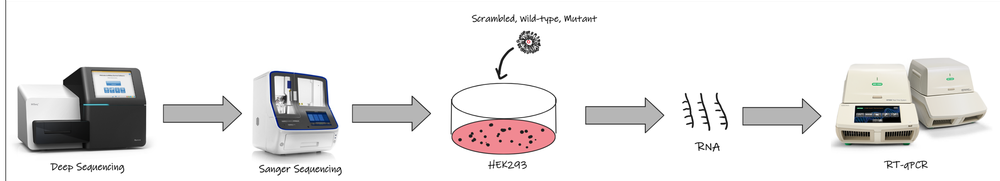

Figure #5. Experimental workflow as described above (ThermoFisher, MiSeq, BioRad)[6-8]

Results

- We identified 5 mutations within the ultra-conserved region (5578) containing the miR-142 sequence among CLL samples
- One notable mutation occurred in the CNNC motif, a known SRSF3 binding site for DROSHA recruitment, 6 nucleotides outside the miR-142 sequence, therefore likely playing a role in miR-142 processing[9]

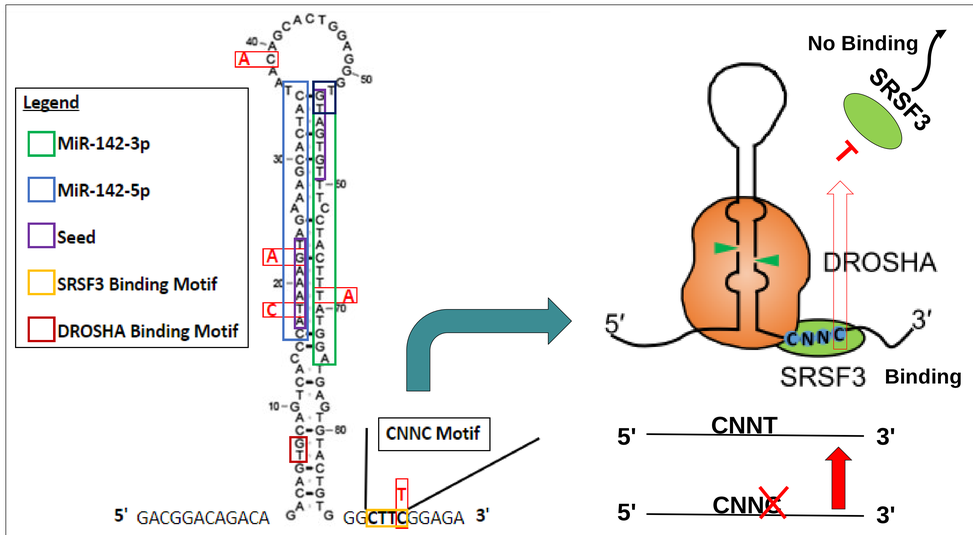

Figure #6. Location of mutations found in ultra-conserved region 5578 with the CNNC motif mutation in CLL, shown with the secondary structure of miR-142 (left) and the involvement of SRSF3 binding at the CNNC motif for subsequent DROSHA cleavage and processing of miRNA into mature forms (right) (USEast Ensemble, Kim *et al.*)[9-10]

- Our *in vitro* assays demonstrated the mutation 6 nucleotides downstream of miR-142 resulted in aberrant downregulation of miR-142-3p levels in HEK293 cells

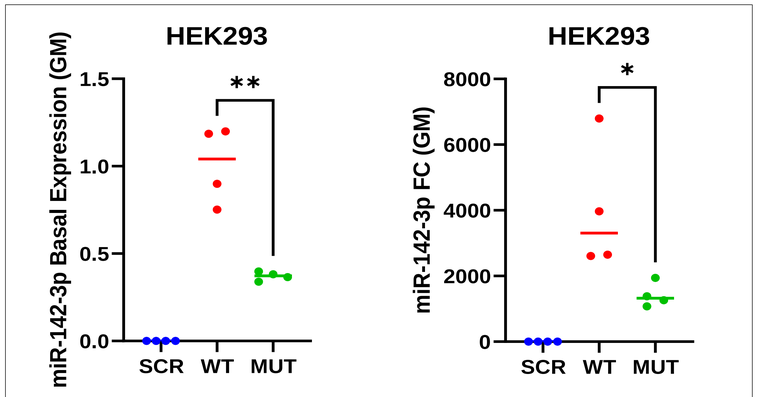

Figure #7. MiR-142-3p basal expression levels (left) and fold changes (right) in HEK293 cells that were non-transfected (NT) or transfected with miR-142 scrambled (SCR), wild-type (WT), or mutated downstream at 6th nucleotide (MUT) normalized to the geometric mean of U6 snRNA and RNU48.

Conclusions

- We confirmed that the mutation 6 nucleotides downstream of miR-142 impaired miR-142-3p processing
- The presence of this mutation in CLL likely upregulates miR-142-3p targets, such as *WASL*, *MRFAP1*, and *RPS11*, many of which are linked to cancer[1,2]
- Other mutations identified could also play a role in miR-142 processing, but further investigation is necessary.

Acknowledgements

Special thanks to Recep Bayraktar and Dr. George A. Calin for their continued mentorship and support.

Dr. Calin is the Felix L. Haas Endowed Professor in Basic Science. Work in Dr. Calin's laboratory is supported by National Institutes of Health (NIH), National Center for Advancing Translational Sciences grant UH3TR00943-01 through the NIH Common Fund, Office of Strategic Coordination, National Cancer Institute (NCI) grants 1R01 CA182905-01 and 1R01CA222007-01A1, a National Institute of General Medical Sciences (NIGMS) 1R01GM122775-01grant, a U54 grant #CA096297/CA096300–University of Puerto Rico and The University of Texas MD Anderson Cancer Partnership for Excellence in Cancer Research 2016 Pilot Project, a Team DOD (CA160445P1) grant, a Chronic Lymphocytic Leukemia Moonshot Flagship project, the UT MD Anderson Cancer Center Duncan Family Institute for Cancer Prevention and Risk Assessment, a Sister Institution Network Fund 2017 grant, and the Estate of C. G. Johnson Jr.

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