



# Epigenetics: Do MicroRNAs Ask the Right Questions?

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CUTTING EDGE of **TRANSPLANTATION**

**TRANSPLANT SUMMIT** 2019

***NO SIZE FITS ALL:** Uncovering the  
Potential of Personalized Transplantation*

# Disclosures

- Grant Support: Merck, Haemonetics, Medtronic, American Heart Association / Enduring Hearts Scientist Development Grant
- Consultant: NuPulse CV, Ortho Clinical Diagnostics

# Mission Statement

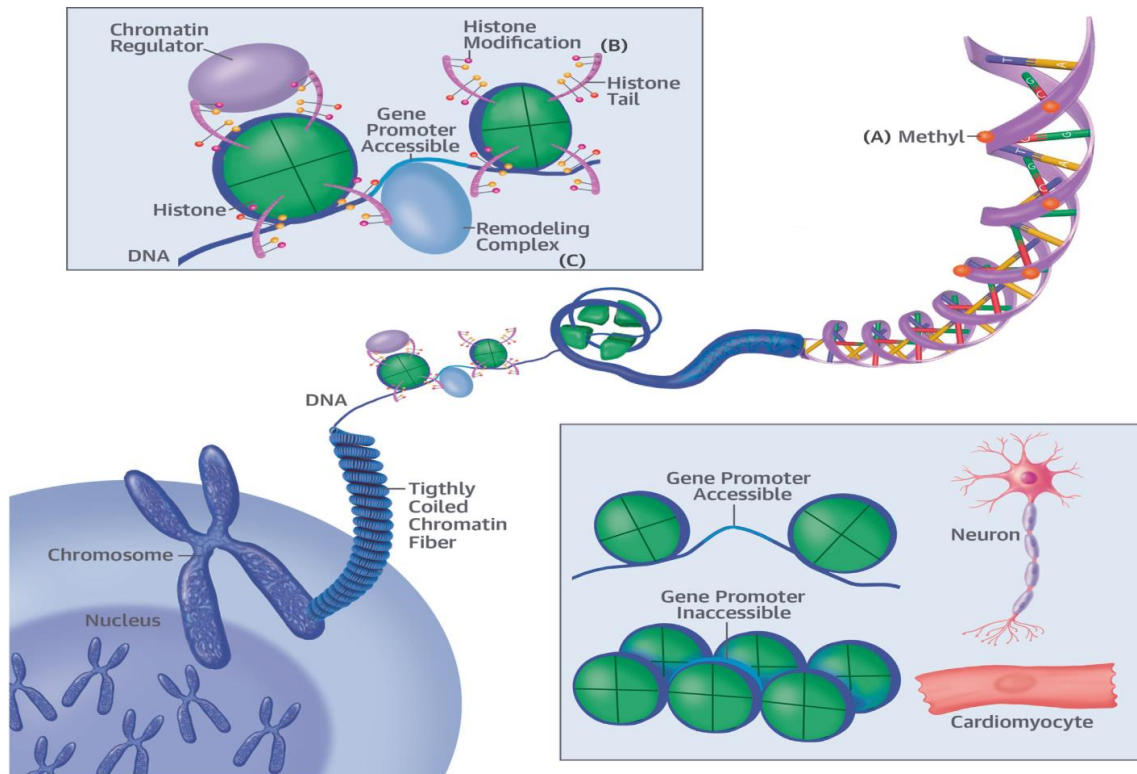
*Develop a blood-based, biomarker that accurately predicts risk for rejection, diagnoses rejection and the major subtypes (TCMR & AMR), reflects immune system activity (immunosuppression phenomapping), prognosticates long-term risk of graft failure, and ultimately eliminates the need for invasive endomyocardial biopsies.*

# Learning Objectives

1. Describe how microRNAs and other epigenetic mechanisms regulate gene expression.
2. List the characteristics of microRNAs which make them viable circulating and tissue-level biomarkers of allograft rejection.
3. Distinguish how microRNAs can be used to develop novel therapeutic targets for the prevention or treatment of allograft rejection.

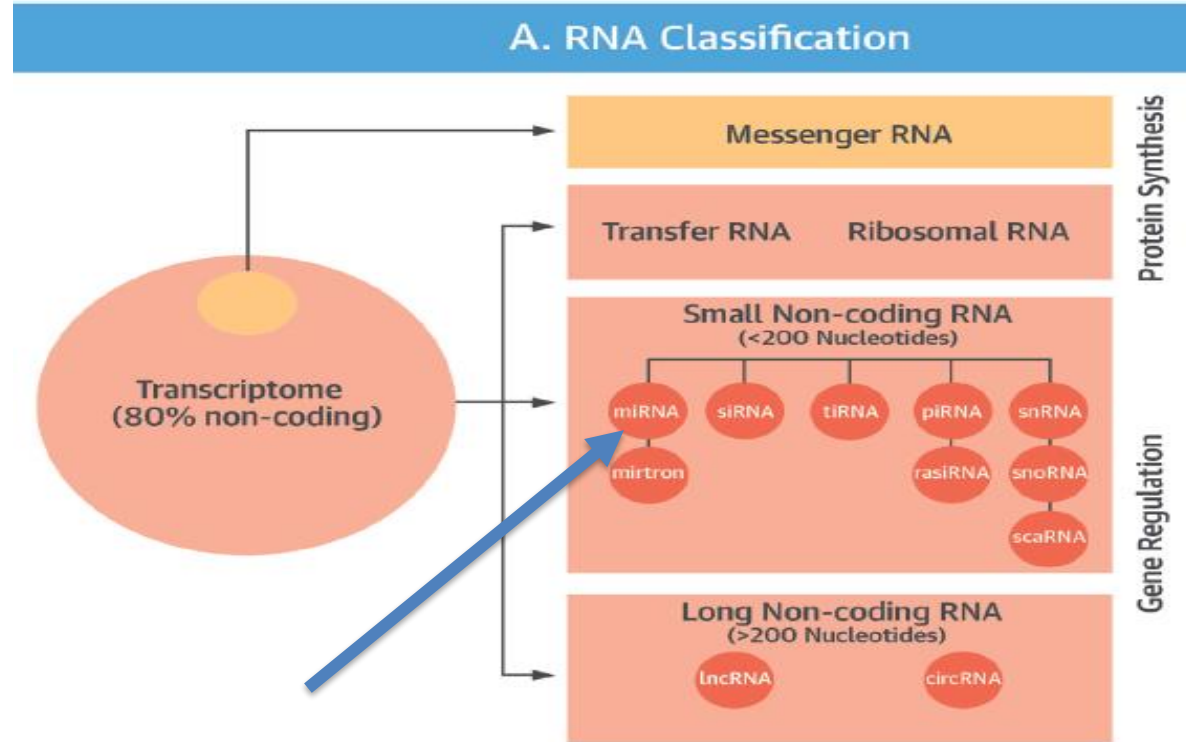
# Epigenetics

- Mendelian Genetics  
→ explains little risk associated with lifestyle, environmental, pharmacologic therapy
- Epigenetics Factors: Methylation, Histone / Chromatin
- Non-coding RNAs (long non-coding RNA and microRNAs)



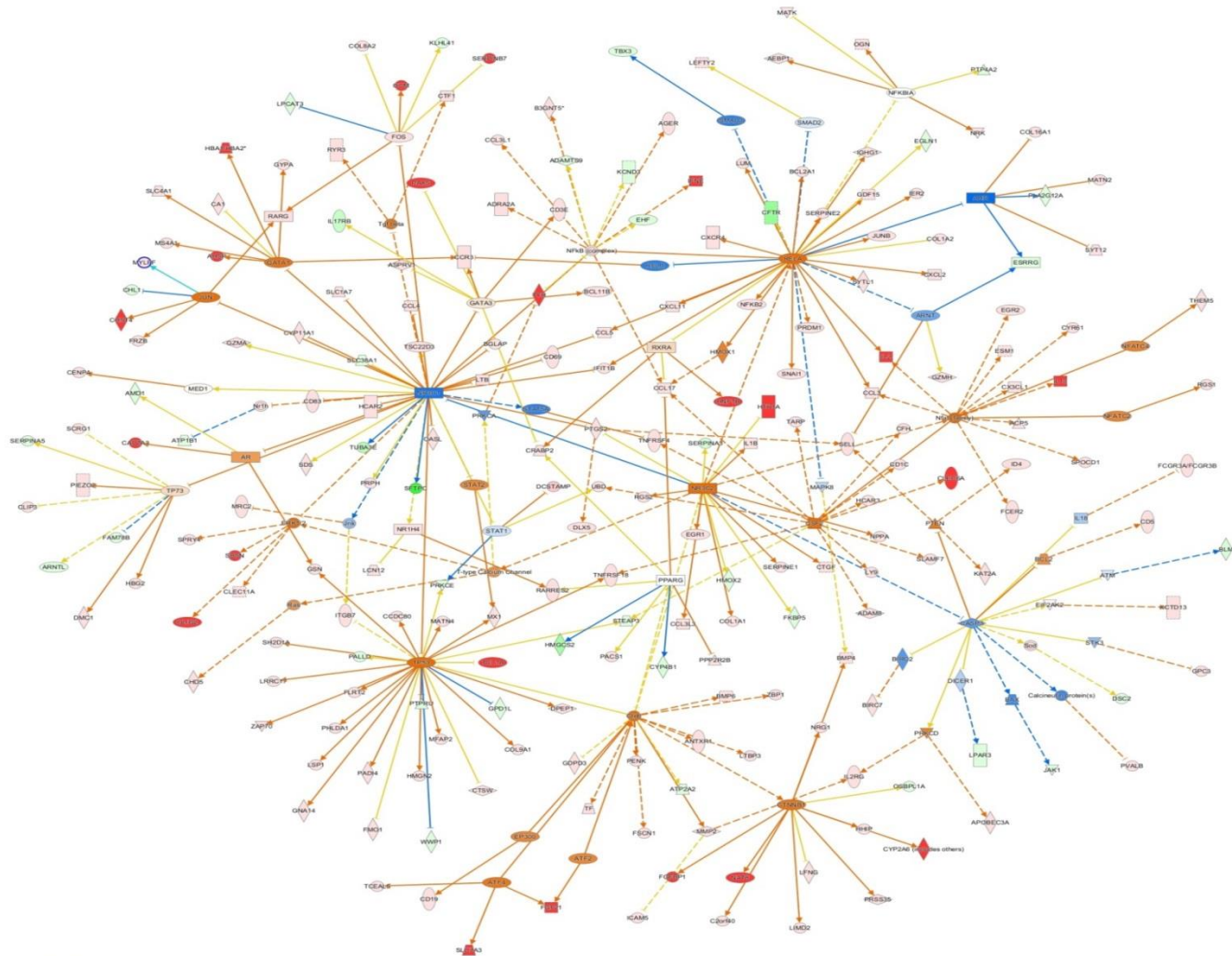
# Non-Coding RNAs

- Variety of non-coding RNA species



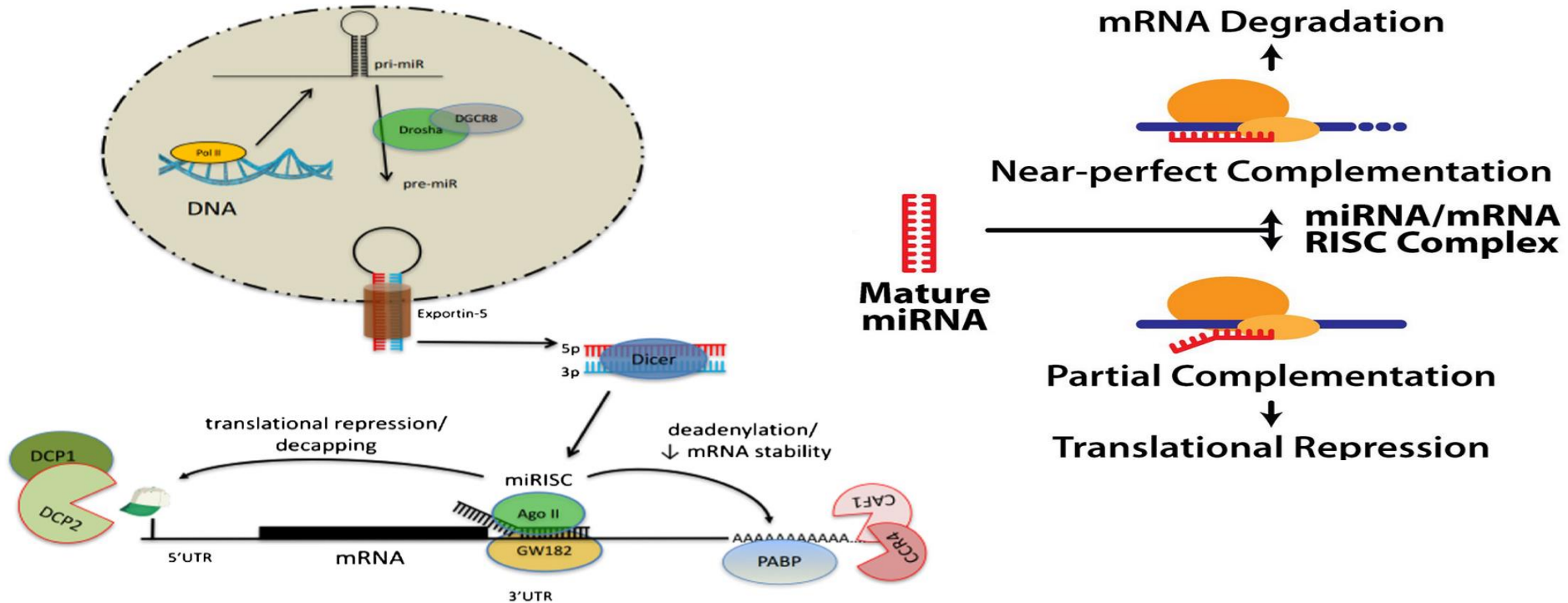


- Small RN
- Non-codi
- Human C
- 2,000
- Highly co
- Suppress
- expressic
- Complim
- multiple



AAAGUA-3'  
 AAAGC-3'  
 AAAGC-3'  
 AAAGUA-3'  
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 1-5'

# MicroRNA Biology



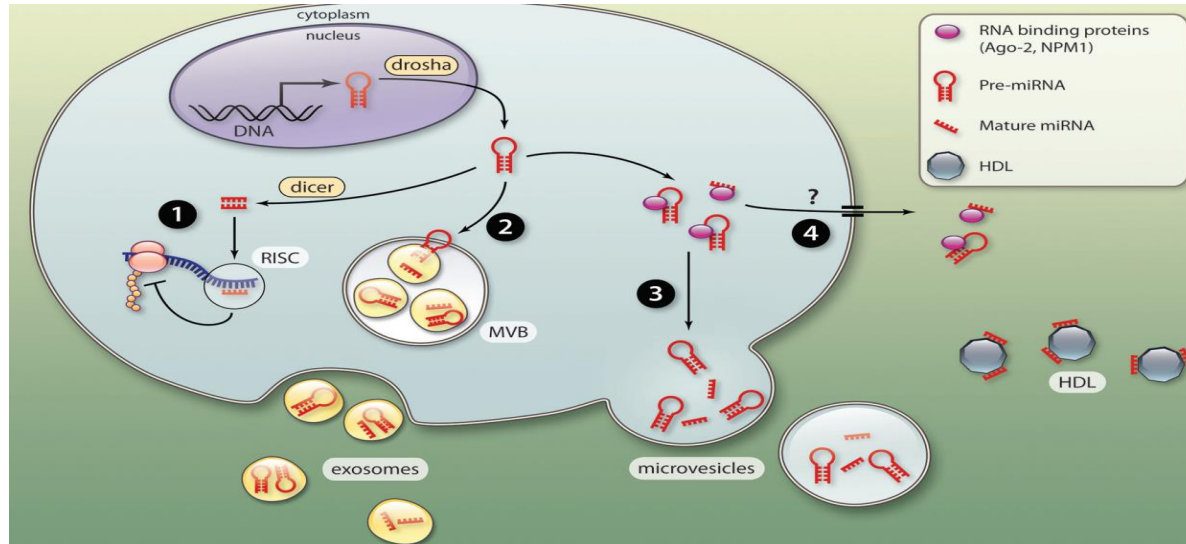


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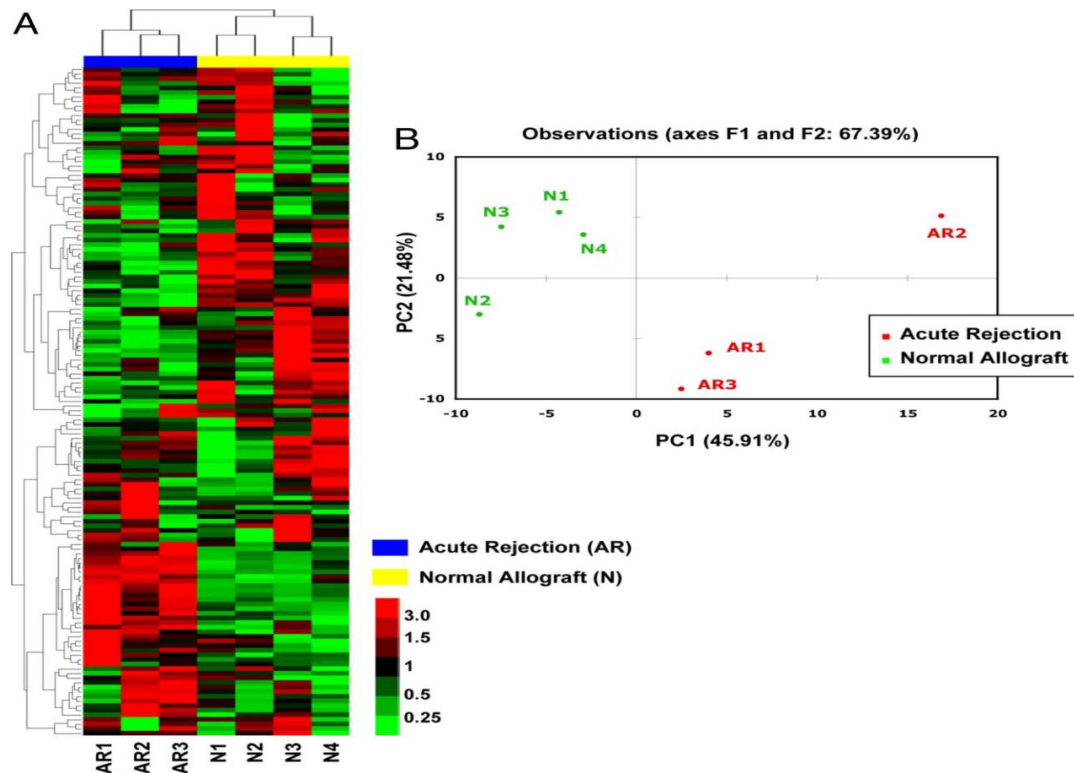
# Biomarker Characteristics

- High stability in plasma:
  - Repetitive freeze-thaw cycles
  - Boiling conditions
  - Across pH ranges
  - At room temperature



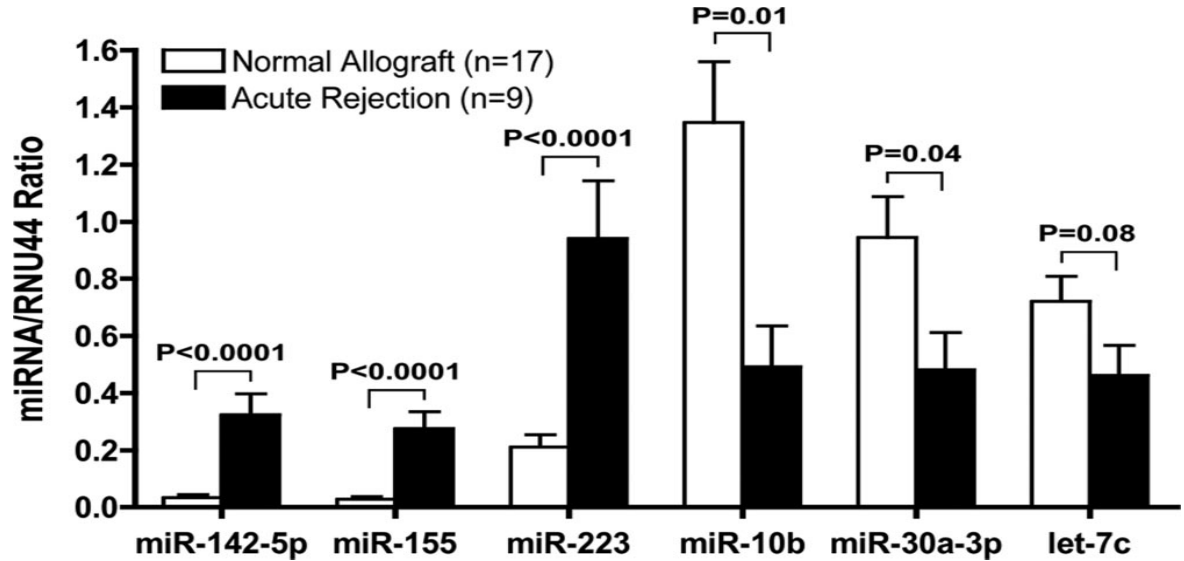
# MiRs as Rejection Biomarkers

- Profiled 365 microRNAs, 174 expressed
- 33 renal allograft biopsies (12 – ACR, 21 – normal)
- Training / Validation sets



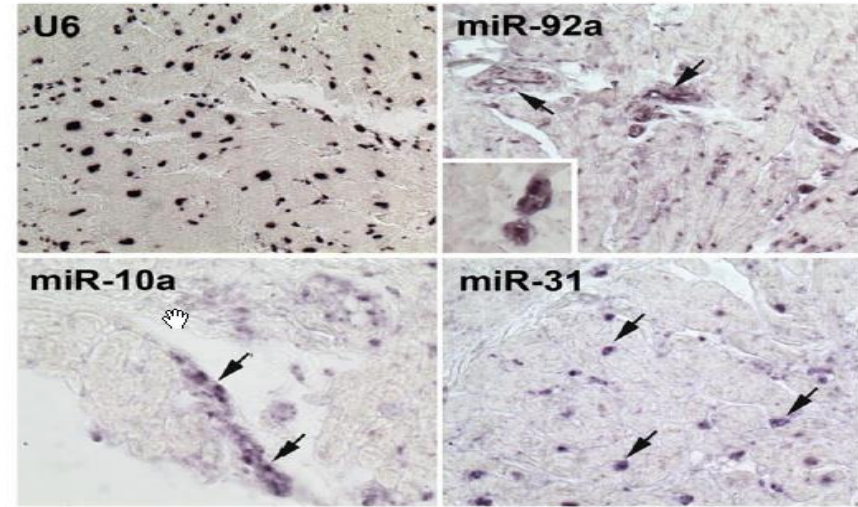
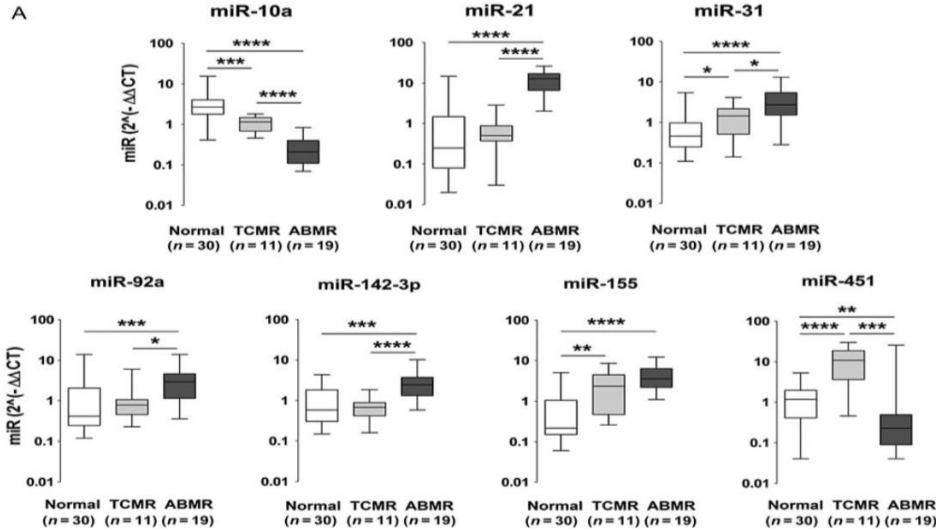
# High Diagnostic Accuracy

- High sensitivity & specificity
- Area Under the Curve (AUC) for:
  - miR-142-5p (0.99)
  - miR-155 (0.98)
  - miR-223 (0.96)



# Cardiac Allograft Rejection

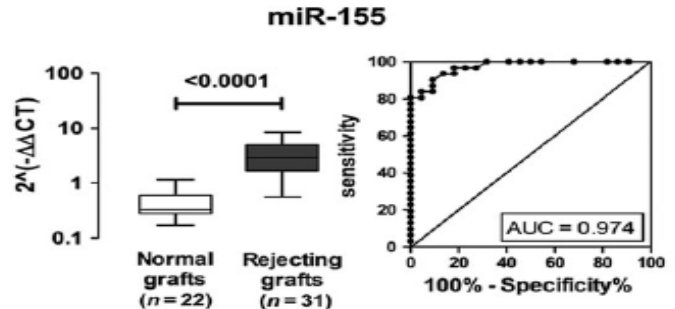
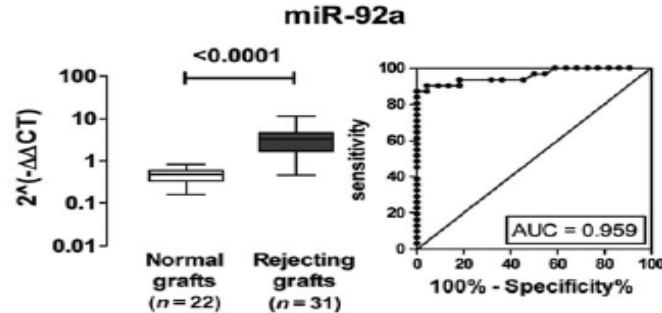
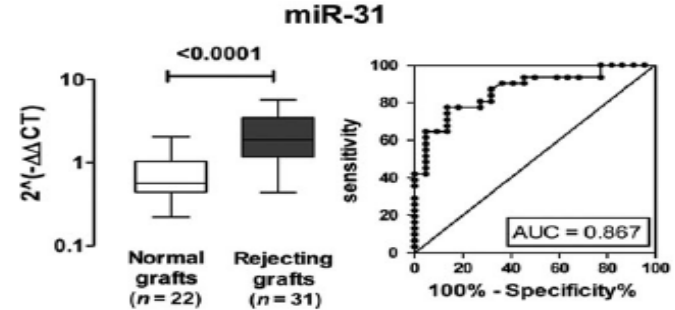
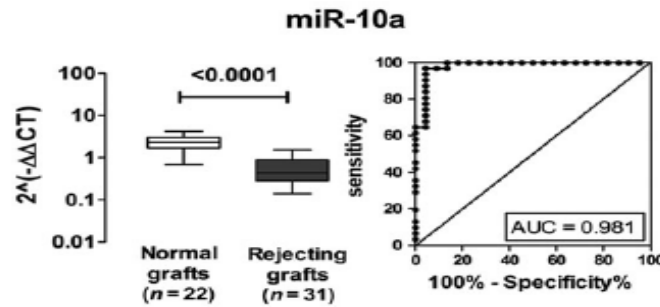
- Myocardial miR analysis, evaluated 14 miRs by PCR
- 113 cardiac transplant recipients (~50% rejection)





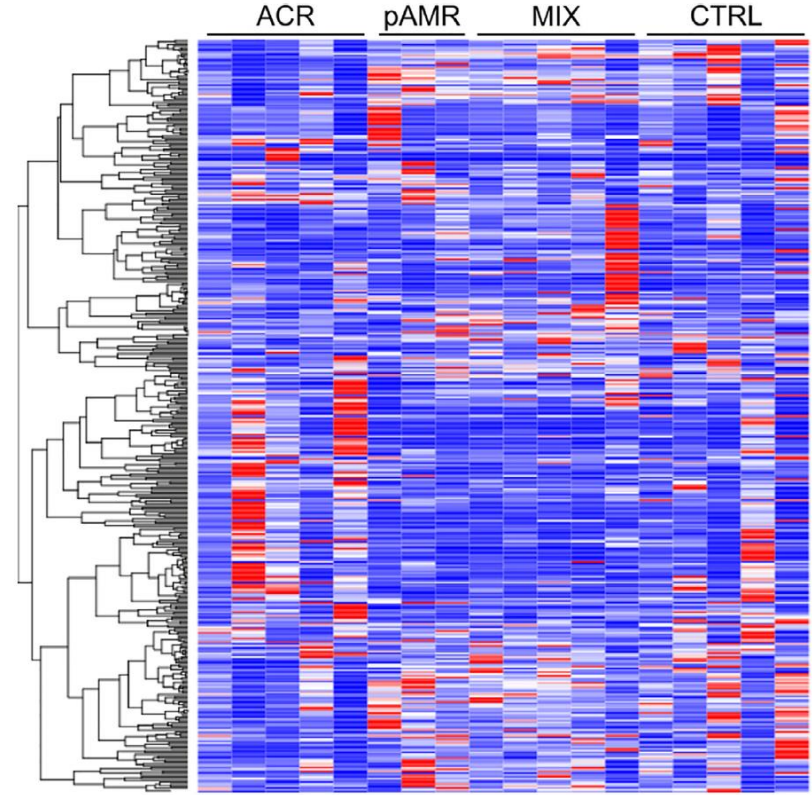
# Tissue v. Serum Analysis

- Patients divided into training and validation set
- Tissue & Serum analysis



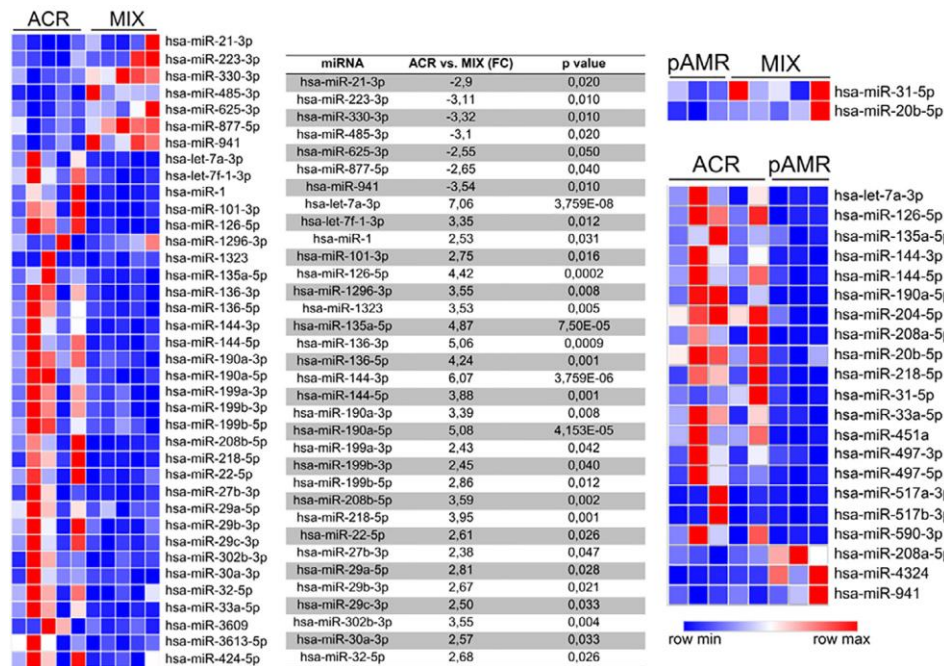
# Cardiac Biopsy MiR Sequencing

- 33 Endomyocardial Biopsies – 20 sequenced
- Candidate miRs, evaluated by PCR in 13 samples
- Paraffin embedded tissues: ACR (n=12), AMR (n=6), MR (n=6) and No rejection (n=9)



# Performance and miR Localization

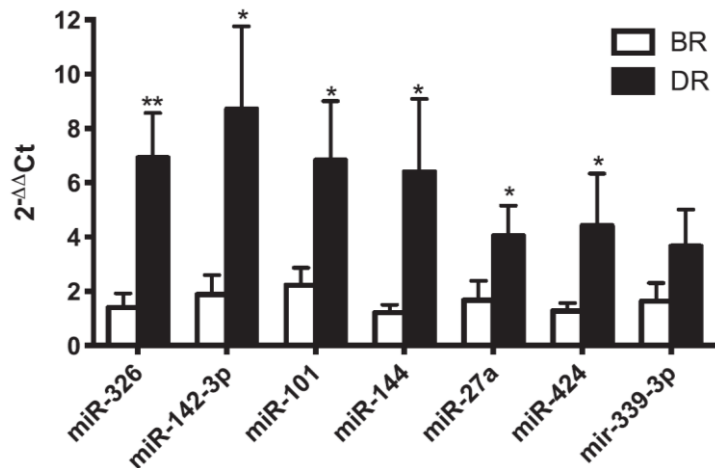
- AMR v ACR  
*20 miRs DE*
- MR v. AMR  
*2 miRs DE*
- MR v. ACR  
*47 miRs DE*



*miRs may aid in more accurate diagnostic classification of endomyocardial biopsies, an even more powerful molecular microscope?*

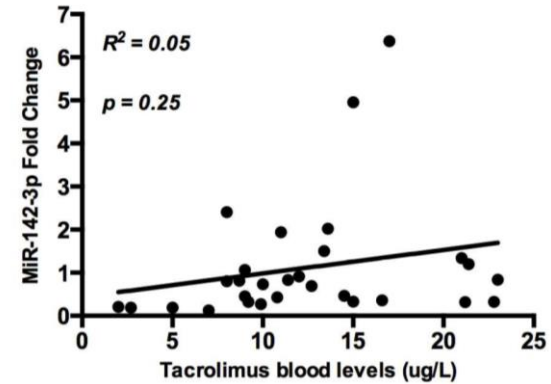
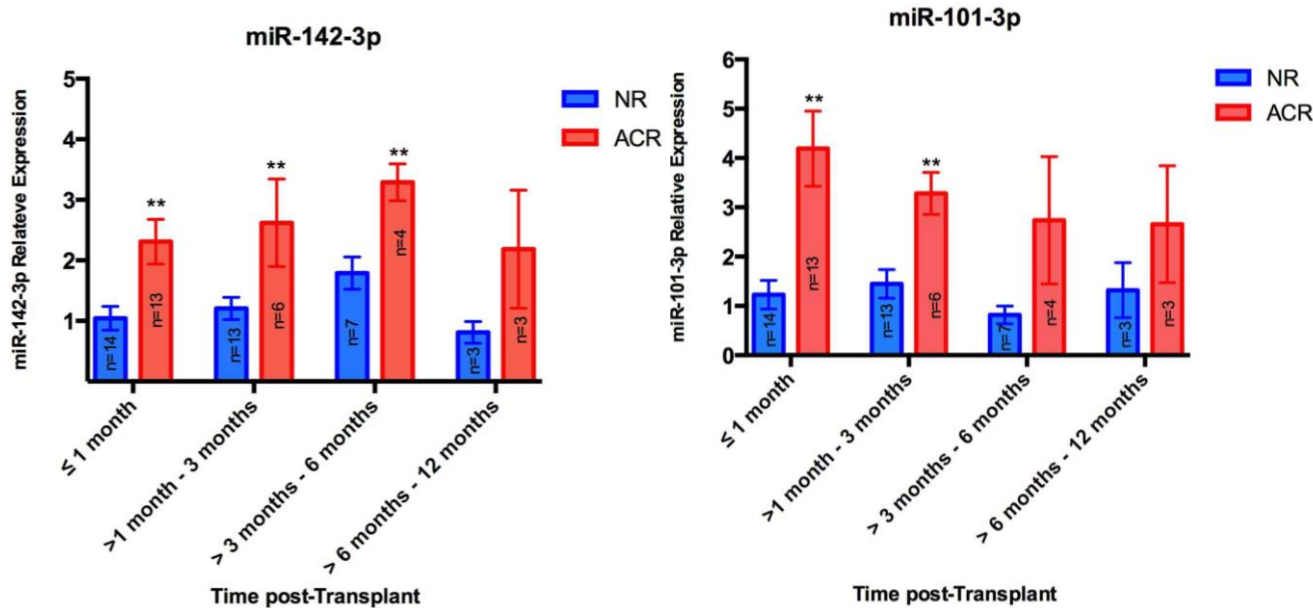
# Predicting Rejection & Assessing Response to Therapy

- 10 patients with rejection
- PCR of samples before, during and after cellular rejection



# Sensitivity of microRNAs Over Time Post-Transplant

- 26 ACR, 37 controls; 7 miRs of interest







American  
Heart  
Association®

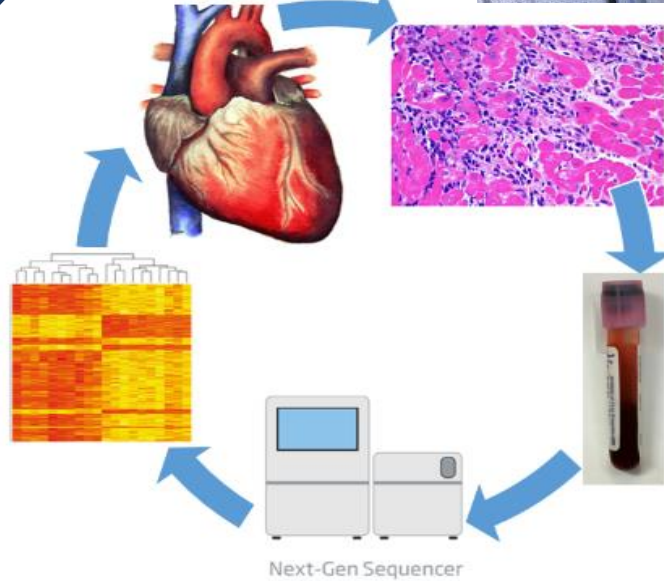


**Enduring Hearts**  
HEALTHY KIDS • STRONGER HEARTS • LONGER LIVES

## Our Work

Why Sequence?:

- Annotate all circulating 2,200 miRs
- Identify miRs associated with multiple transplant co-morbidities



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TRANSPLANTATION

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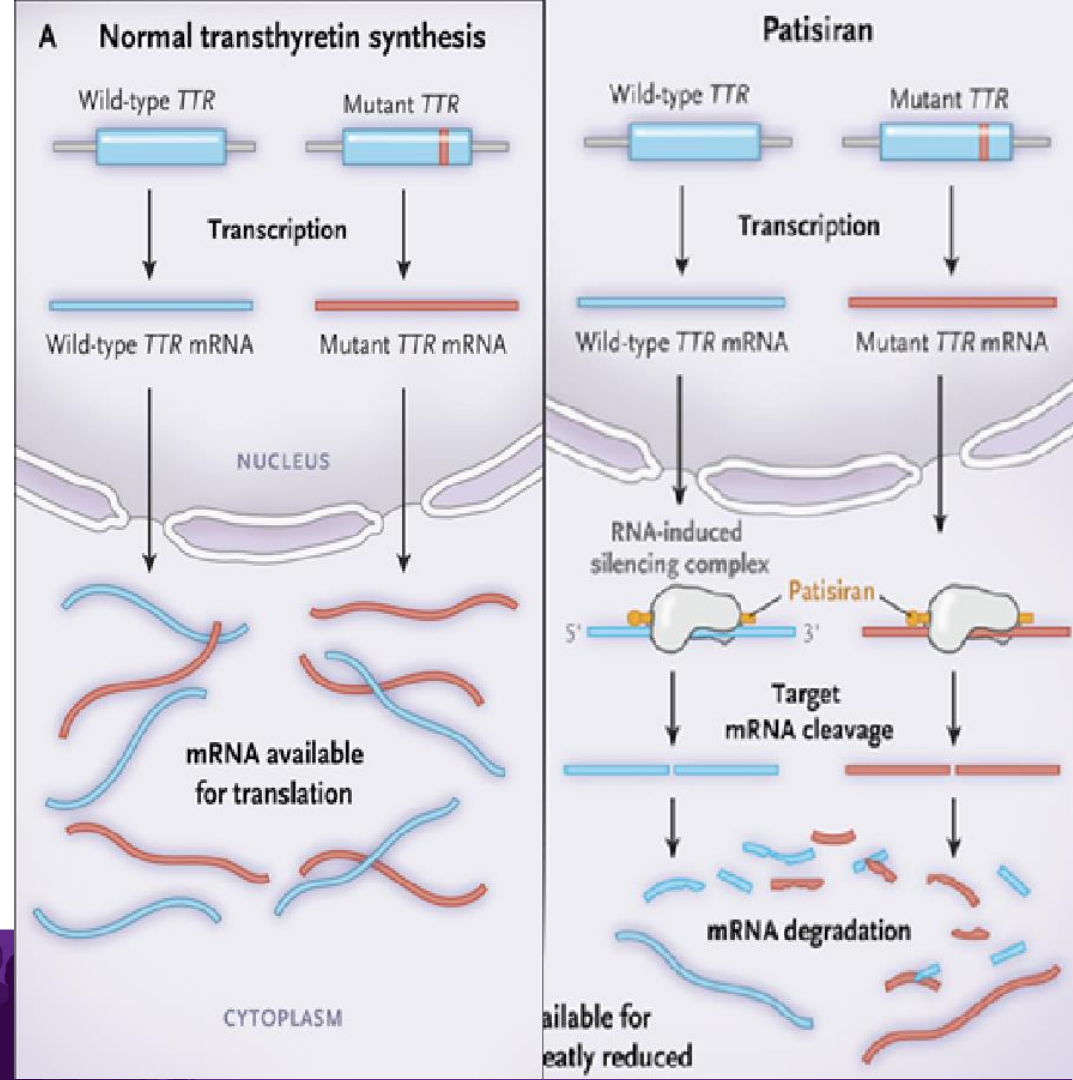
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# Small RNA Therapeutics

- Lack of new immunosuppressant therapies
- microRNAs inhibit gene expression
- miRNA-based therapeutics can be classified as miR mimics and inhibitors (aka antimirs or antagomirs)

# RNA Interference

- Inclisiran example in dyslipidemia
- Patisiran example in TTR Amyloid



# Conclusions

- MicroRNAs suppress gene expression
- Highly stable in the circulation
- Promising biomarkers in tissue and blood for the diagnosis of allograft rejection and its subtypes
- Outside of biomarkers, are potential targets to develop novel oligonucleotide therapies (mimics or antimirs)



# THANK YOU

