micro-RNAs as biomarkers in children who underwent surgery for CHD

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המחלקה לטיפול נמרץ ילדים
בית החולים אדמונד ולילי ספרא לילדים

What will we talk about…

- Congenital heart defects (CHD)
- Scientific background
- miRNAs in CHD
- Lab work
- What's next

Congenital heart defects (CHD)

- Incidence: 8/1000
- Most common birth defect
- Causes:
  - Unknown
  - Infections (e.g. rubella)
  - Medication (e.g. Thalidomide)
  - Alcohol/Tobacco
  - Inbreeding
  - Nutritional status (undernutrition, DM, etc)
  - Genetic – mostly sporadic mutations
- Attitudes toward pregnancy termination
- Cyanotic vs. non-cyanotic

Congenital heart defects (CHD)

- Treatment:
  - Some defects do not need intervention
  - Medications (diuretics, digoxin, etc)
  - Catheter based procedures
  - Heart surgery
  - Heart transplant
- Complications after surgery
  - Leading cause of birth defect related deaths
  - Early mortality – 5-10%
  - SIRS (1/3 of cases)
  - Arrhythmias and heart failure
  - Lung injury

  Neurological and renal complications

The need for early diagnosis

- Early diagnosis of complications may improve treatment and its outcomes
- Today's biomarkers:
  - Biomarkers for myocardial injury:
    - Troponin, CPK, BNP
    - Cardiac miRNA
  - There are no specific inflammatory biomarkers

MicroRNA

- MicroRNAs (miRNAs) are small non-coding RNA molecules
- They consist 19-24 nucleotides
- Constitute 1-3% of the human genome (over a thousand have been identified in human)
- Main role: post-transcriptional regulation
  - Inhibit mRNA translation
  - Promote mRNA degradation

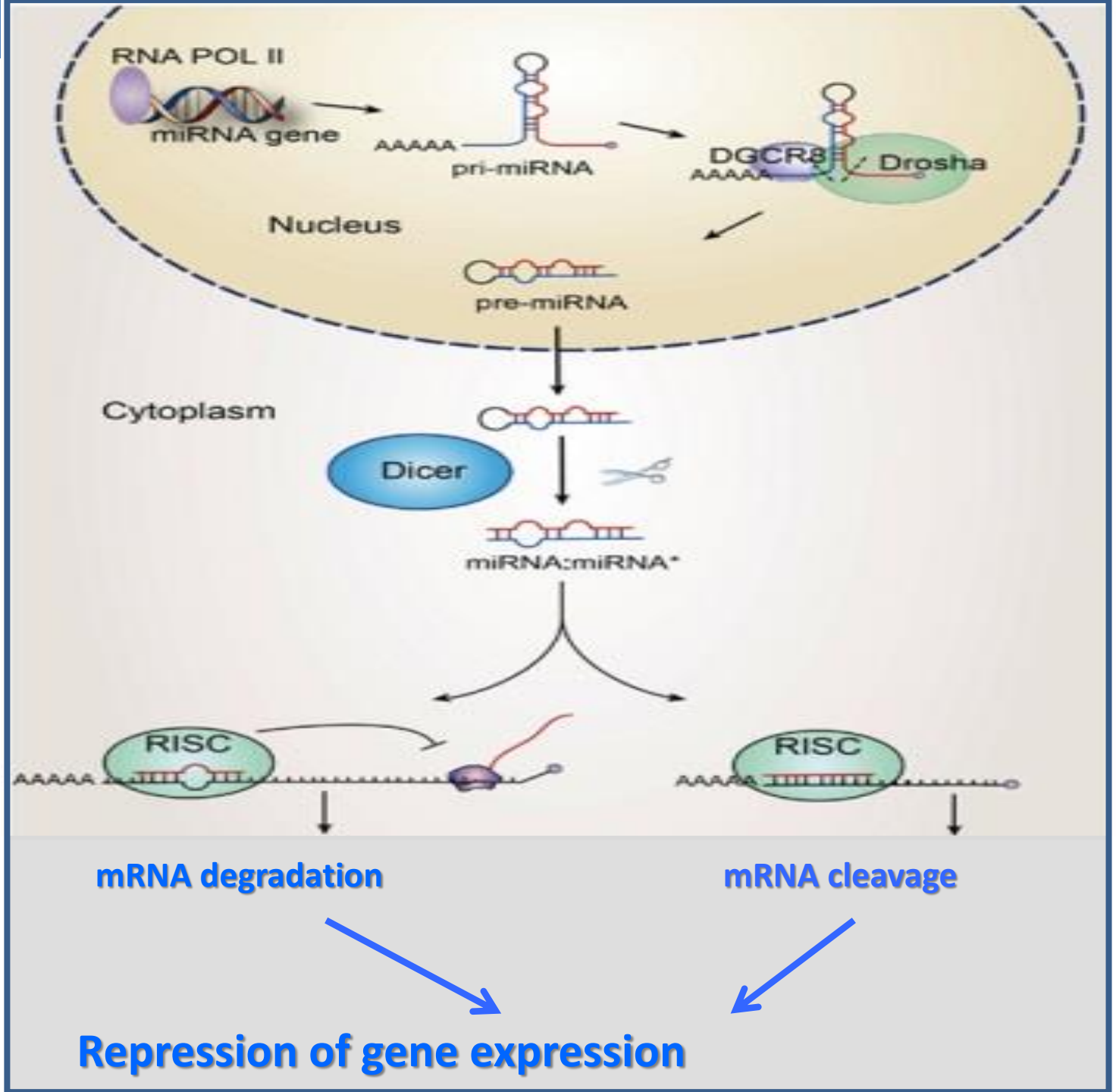
MicroRNA

- Over 50% of human genes are likely regulated by miRNA
- Tissue-specific expression pattern
- Dysregulated miRNA expression:
  - Cancer
  - Inflammatory diseases
  - Autoimmune diseases
- Role in immune system:
  - Immunomodulation and fine-tuning

MicroRNA

- **Circulating miRNAs in the serum:**
  - **Cell damage and cell death**
  - **Cell communication**
  - **High stability**
  - **Ribonucleoprotein complex**
  - **<u>Intercellular communication via extracellular vesicles (EVs)</u>**
    - **Ectosomes**
    - **Exosomes**
    - **Apoptotic bodies**
- **Also found in urine, saliva, CSF and breast milk**
  - **Breast milk miRNAs may have a role in immunoregulation**

MicroRNA – biogenesis



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A promising field of research…

- Potential therapeutic use

Requirements:

1. **Specificity of miR to pathology**
2. **No/minimal side effects**
3. **Bio- availability**
4. **Cost effectiveness**

No micro-RNA-based drug is in the market…yet

A promising field of research…

- Specific biomarkers

Requirements:

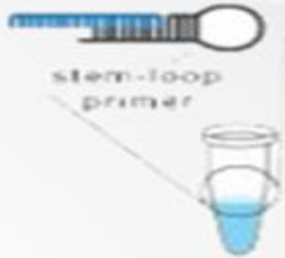
1. Specificity of miR to pathology
2. Significant change in expression
3. Circulating miRs
4. Stable in blood
5. Reliable testing
6. Simple, quick and reproducible diagnosis

Bonus: may predict the prognosis

1. Blood sample collection

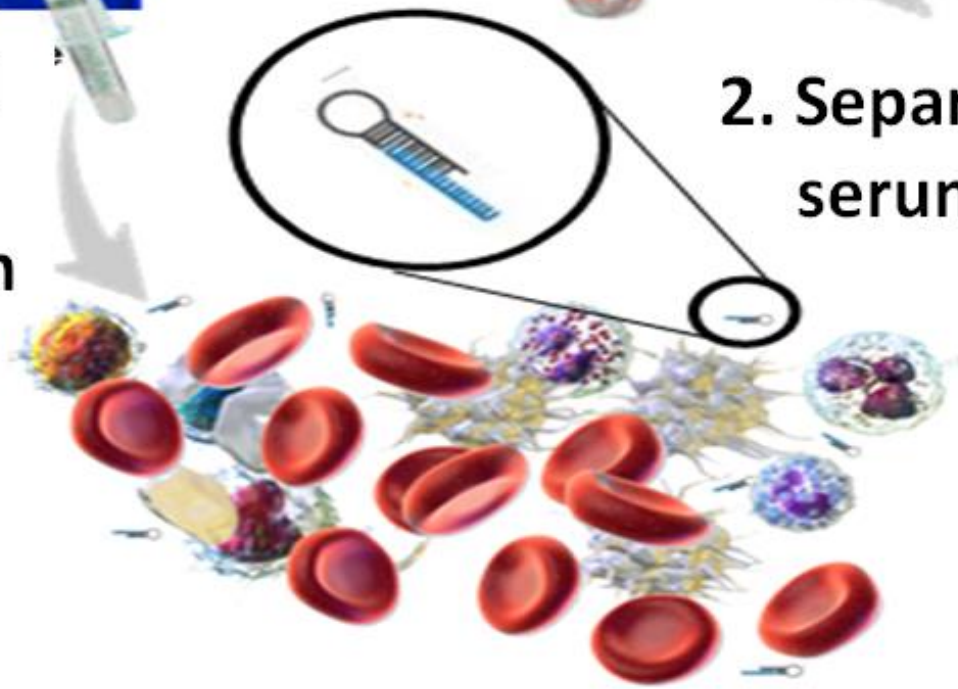
3. RNA extraction

4. cDNA enrichment



5. Setup quantitive real-time PCR plate

2. Separate serum



6. Run RT-PCR reaction

7. Analyze data

Immunomodulatory miRNAs as biomarkers in pediatric patients after cardiac surgery

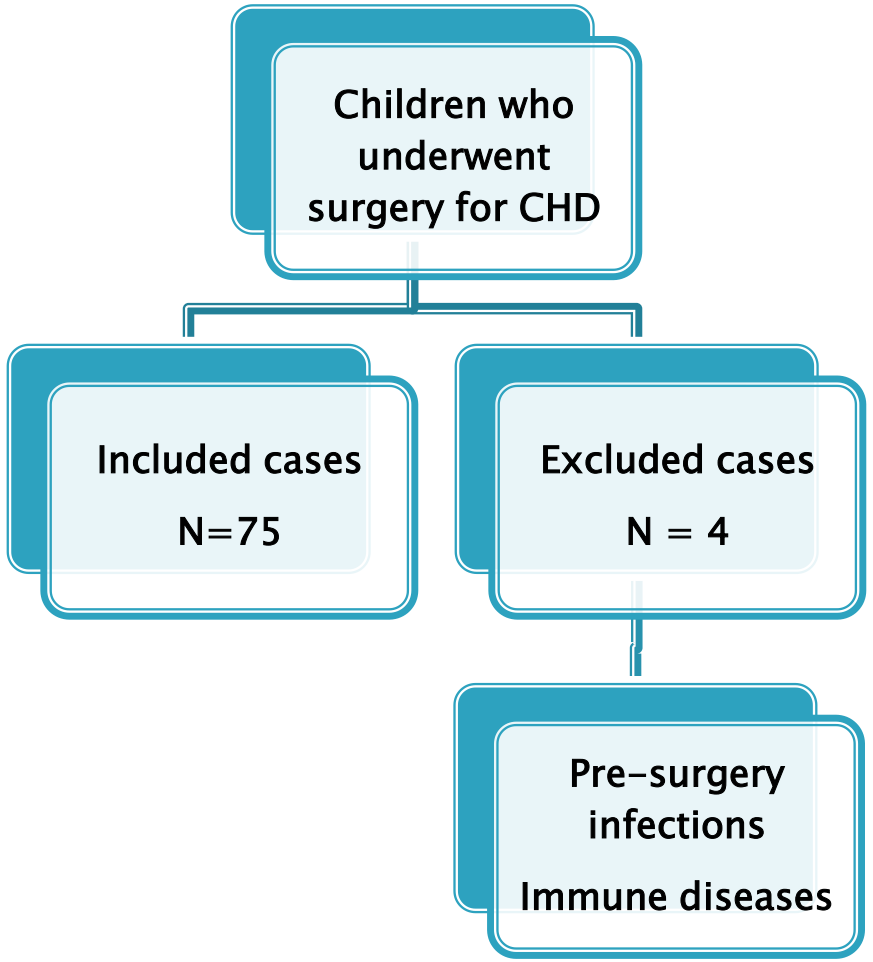
Mentors: Prof. Gidi Paret
Dr. Yael Nevo-Caspi

Or Bercovich
Pediatric intensive care unit –
Sheba Medical Center

Objective

- **Examination of the association between the expression of immunomodulatory miRNAs and the inflammatory response following surgery for CHD**
- **Development of a diagnostic tool that will improve medical management and outcome following surgery for CHD**

Research pupolation



Immunomodulatory miRNAs in CHD

- A better understanding of the inflammatory response to cardiac surgery may be the key to development of successful strategies to minimize patient morbidity and mortality
- Inflammatory response to cardiac surgery:
  - Prevent infections
  - Wound healing
- Validated biomarkers are essential for guiding drug therapy

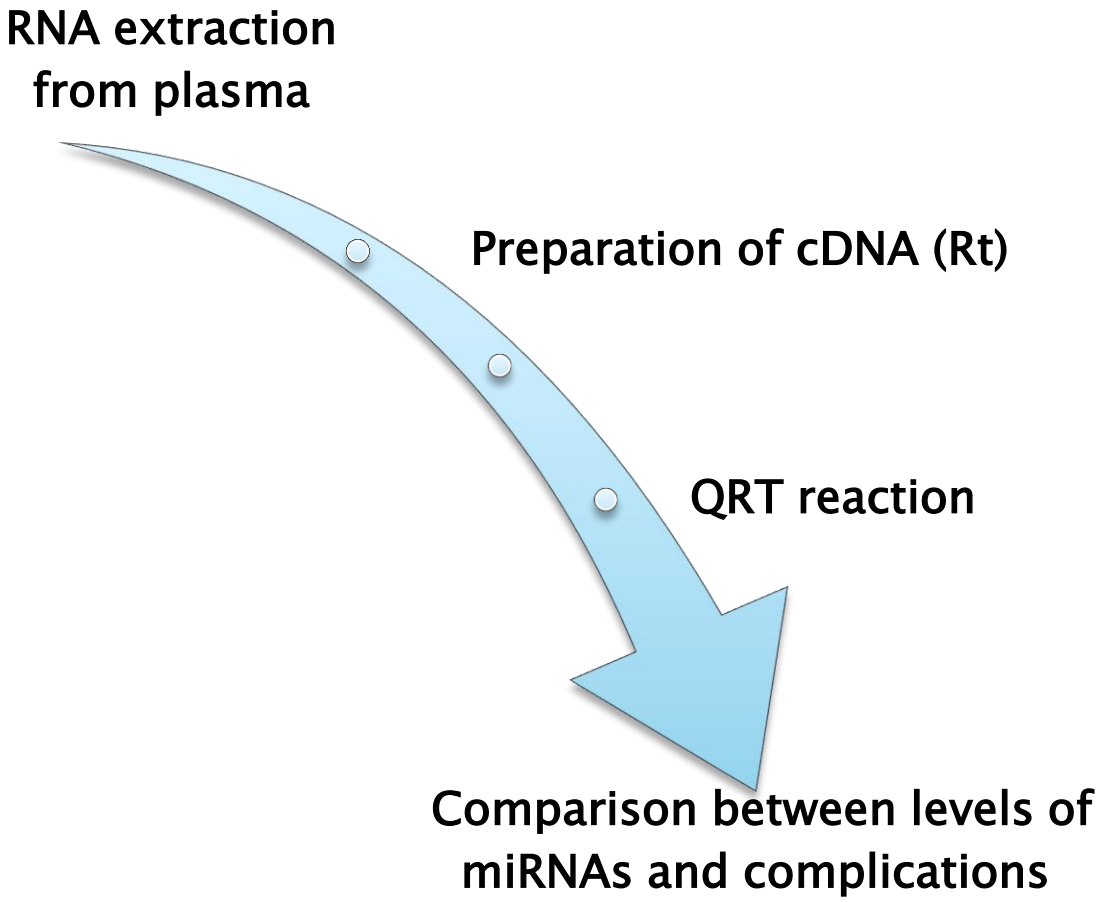
Immunomodulatory miRs in CHD

- miRNA 155 & 146a –
  - Found in EVs
  - Regulate many aspects of the immune response
- miRNA 146a and 146b –
  - Low expression may cause a hyperactive immune response

Immunomodulatory miRs in CHD

- miRNA 21 –
  - highly expressed in the fetal heart
  - Promote inflammatory mediators
  - Important marker of immune cell activation in multiple contexts

Lab work


RNA extraction
from plasma
Preparation of cDNA (Rt)
QRT reaction
Comparison between levels of
miRNAs and complications

Lab work

- We test by RQ PCR 4 miRNAs for each patient
- Each miRNA is tested in 4 different times:
  - 0h – before surgery
  - 6h
  - 12h
  - 24h
- RQ calculation:
  - High expression of a specific miRNA → Fluorescence in an earlier cycle (and vice versa)
  - miRNA expression after surgery of each child is compared to 0h

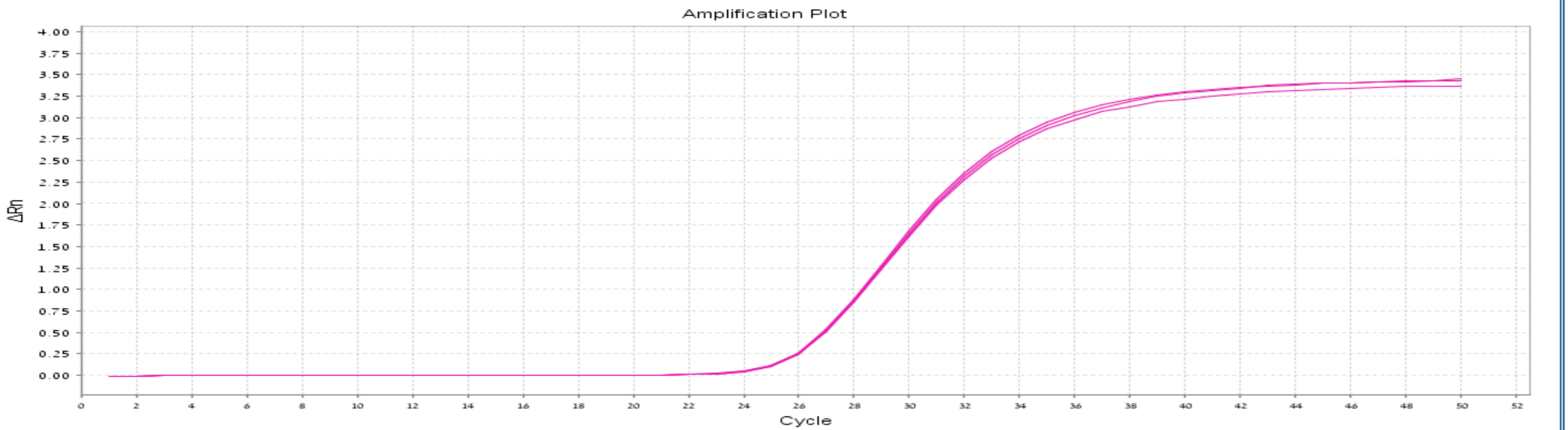
Experiment: OR-1 041216 Type: Comparative CT (ΔΔCT) Reagents: TaqMan® Reagents Analyze Analysis Settings

Amplification Plot

Plot Settings

Plot Type: ΔRn vs Cycle Graph Type: Linear Plot Color: Well

Save current settings as the default



Legend

A B C D E F G H

Options

Target: All Threshold: Auto Auto Baseline

Show: Threshold Baseline Start: Well Target Baseline End: Well Target

Analysis Summary: Total Wells in Plate: 96 Wells Set Up: 84 Wells Omitted Manually: 0 Wells Flagged: 0 Wells Omitted by Analysis: 0 Samples Used: 12 Targets Used: 4

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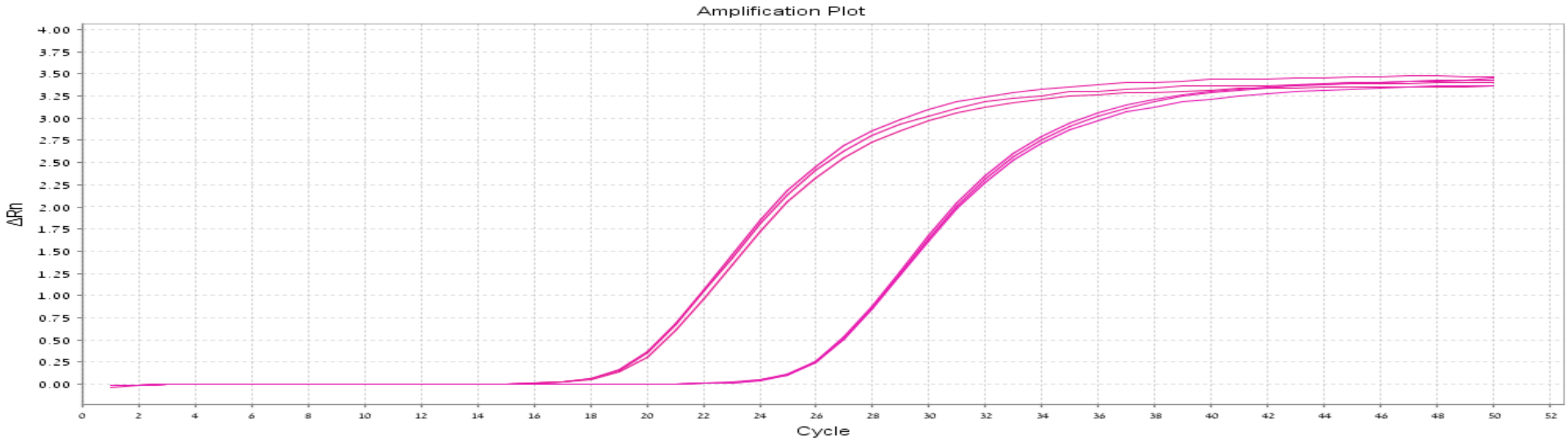

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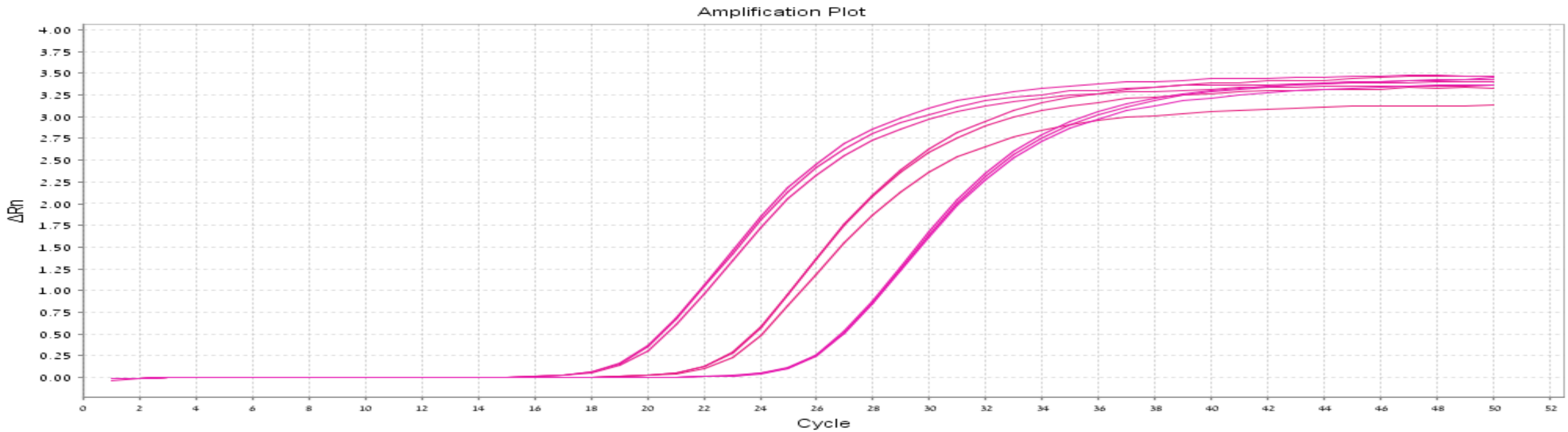

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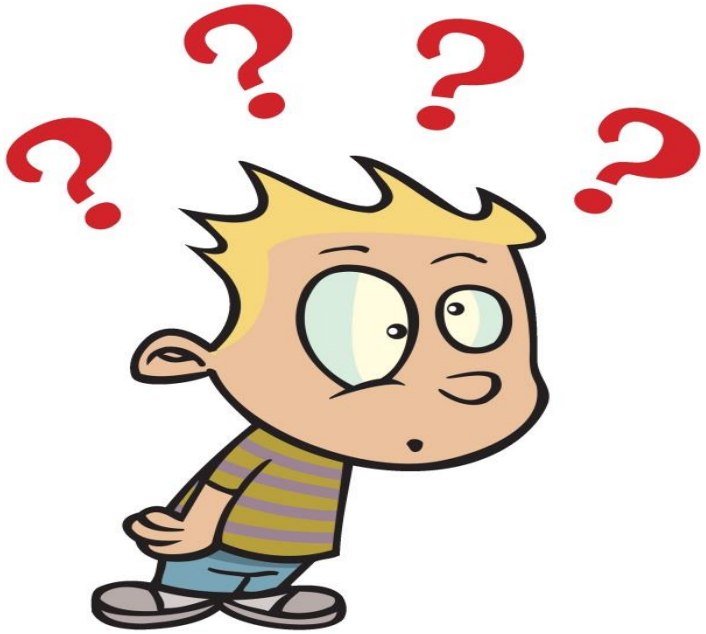
What's next

- Continue lab work
- Statistical analysis
- Writing

Summary

- Immunomodulatory miRNAs may help physicians in the future to take preventive steps against expected complication

Questions



Thank you!

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MicroRNAs as Biomarkers for Brain Damage Following Cardiac Surgery in Children

Mentors:
Dr. Yael Nevo-Caspi & Prof. Gidi Paret

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BACKGROUND

- Brain injury is the most prevalent post-operative complication (40-70%)
- Wide range of neurological injuries
- Mechanism: brain hypoxia-ischemia
  - Surgery induced stress and inflammation
  - Weak heart due to CHD
  - Cardio-pulmonary by-pass (???)

Current testing methods

Pediatric Cerebral Performance Category (PCPC)-

cognitive function following a critical illness or injury

Scale of 1-6

Pediatric Stroke Outcome Measure (PSOM)-

5 subscales: right+ left sensorimotor, language production+ comprehension, and cognitive function

Scale of 0-2 per function

Disadvantages:

- Gross assessment based on observer's impression
- Insignificant while child is unstable ⟶ late diagnosis
- Do not indicate prognosis

Aim of study

To discover a new biomarker that will enable early diagnosis of brain damage and it's prognosis

Why?

1. **To allow neuroprotective therapy**
   (=hypothermia ???, adiponectin etc.)
2. **To ensure close lookup on brain-functions**
3. **To adjust additional therapy and recovery plans**

Research population

Blood samples were collected at 0h, 6h, 12h & 24h from surgery

Children who underwent surgery for congenital heart defects

N=40

No post-operative neurological damage

N=20

Post-operation neurological damage

N=20

Deciding on miRs was difficult…

Requirements	Difficulties
Brain specific/ brain enriched miR	70% of known miRs are expressed in the brain
Present in serum	Some promising miR are un-detectable in serum
Dysregulation of expression due to neurological damage	Inconsistent trends of most miRs in different studies

Micro-RNA 124

- A significant brain-enriched miR
- Expressed in various neurological processes:
  neurodevelopment and differentiation, neuronal degradation & stress
- The most researched brain-specific miR
- To our needs:
  - Significantly elevated after 6h in stroke patients
  - extent of elevation strongly correlates with neurological outcome in rat study

Micro-RNA 107

- A brain enriched miR
- Involved in different neurological processes
- To our needs:
  - Present in plasma of stroke patients
  - Studies showed significant elevation of its serum-levels within 6 hours of ischemic stroke*

Initial results

Blood samples of 3 control infants were tested for levels of miR-107 & miR-124:

- **2 had a similar trend of reduction in miRs levels**
- **1 had opposite results:**
  - **basal level was significantly higher**
  - **the level of serum-miRs increased after surgery**

His medical file revealed he had congenital hydrocephalus.

What lies ahead…

Better assess the clinical background and post-operative status

More lab work ☺

Statistical analysis

Find correlations!

Questions I would like to answer…

- What is the miRs trend in healthy and injured infants?
- When is the best time to withdraw blood, so that the miR will be diagnostic?
- Is the trend of dysregulation consistent in all CHD types?
- Are these miRs suitable biomarkers for neurological deterioration in infants with preliminary brain damage?
- And more to come….

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