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

mentors:
Dr. Yael Nevo-Caspi & Prof. Gidi Paret
Or Bercovich
Liat Mor
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

- Intercellular communication via extracellular vesicles (EVs)
  - Ectosomes
  - Exosomes
  - Apoptotic bodies

- Also found in urine, saliva, CSF and breast milk

- Breast milk miRNAs may have a role in immunoregulation
MicroRNA - biogenesis
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

2. Separate serum

3. RNA extraction

4. cDNA enrichment

5. Setup quantitative real-time PCR plate

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
Children who underwent surgery for CHD

Included cases
N=75

Excluded cases
N = 4

Pre-surgery infections
Immune diseases
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
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
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 $\Rightarrow$ Fluorescence in an earlier cycle (and vice versa)
  ◦ miRNA expression after surgery of each child is compared to 0h
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!
MicroRNAs as Biomarkers for Brain Damage Following Cardiac Surgery in Children

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

**Pediatric Stroke Outcome Measure (PSOM)** -

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

Children who underwent surgery for congenital heart defects
N=40

No post-operative neurological damage
N=20

Post-operation neurological damage
N=20

Blood samples were collected at 0h, 6h, 12h & 24h from surgery
Deciding on miR*s was difficult...

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Difficulties</th>
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<tbody>
<tr>
<td>Brain specific/brain enriched miR</td>
<td>70% of known miR*s are expressed in the brain</td>
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<tr>
<td>Present in serum</td>
<td>Some promising miR are un-detectable in serum</td>
</tr>
<tr>
<td>Dysregulation of expression due to neurological damage</td>
<td>Inconsistent trends of most miR*s in different studies</td>
</tr>
</tbody>
</table>
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...

More lab work 😊

Better assess the clinical background and post-operative status

Find correlations!

Statistical analysis
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….
Thank you