MicroRNAs in cardiovascular disease: Role in health and disease, role as biomarkers
**MicroRNAs** ( abbreviated **miRNA** ) are small non-coding RNA molecules (containing about 22 nucleotides) found in plants, animals and viruses.

They function in RNA silencing and post-transcriptional regulation of gene expression.

miRNAs function via base-pairing with complementary sequences within mRNA molecules.

As a result, these mRNA molecules are silenced, by one or more of the following processes:

1. Cleavage of the mRNA strand into two pieces
2. Destabilization of the mRNA
3. Less efficient translation of the mRNA into proteins by ribosomes
1. A protein called exportin-5 transports a hairpin primary microRNA (pri-miRNA) out of the nucleus.

2. An enzyme called dicer (not shown) trims the pri-miRNA and removes the hairpin loop, leaving a double stranded microRNA duplex molecule.

3. In plant cells, the microRNA is usually perfectly complementary to its target mRNA molecule. The microRNA will bond with it and cause the mRNA to break down.

4. In animal cells, the microRNA nucleotides typically don’t pair up with the mRNA nucleotides as well. Their base pairing often follows a pattern though.

5. The microRNA-protein complex’s presence blocks translation as well as speeding up deadenylation (breakdown of the Poly-A tail), which causes the mRNA to be degraded sooner and translated less.
MicroRNA
MicroRNAs in Heart Disease

Types of heart disease

- Valve disease
- Aneurysm
- Coronary artery disease
- Cardiac arrhythmia
- Heart failure
- Cardiomyopathy
- Pericarditis
Myocardial Infarction

Block in Artery

Muscle Damage

Normal
Heart Failure

Heart muscle pumps blood out of the left ventricle.

Weakened heart muscle cannot pump enough blood.

en.wikipedia.org/wiki/Myocardial_infarction
Journal of Applied Physiology Published 1 June 2007 Vol. 102 no. 6, 2104-2111 DOI: 10.1152/japplphysiol.00033.2007
http://nyheartfailure.com/heart-failure
MicroRNA in Acute Coronary Syndrome

MicroRNA in Acute Coronary Syndrome

Table. Characteristics of Patients With Non-ACS and ACS

<table>
<thead>
<tr>
<th></th>
<th>Non-ACS (n=42)</th>
<th>ACS (n=29)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>69.2±2.2</td>
<td>69.7±2.4</td>
<td>0.7566</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>24 (57.1)</td>
<td>23 (79.3)</td>
<td>0.0622</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>121±3</td>
<td>130±5</td>
<td>0.1486</td>
</tr>
<tr>
<td>Diastolic</td>
<td>67±2</td>
<td>77±</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>76.8±3.9</td>
<td>77.4±</td>
<td></td>
</tr>
<tr>
<td>Physical data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>167.9±1.7</td>
<td>168.3±</td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>57.2±2.2</td>
<td>62.9±</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.2±0.7</td>
<td>23.5±</td>
<td></td>
</tr>
</tbody>
</table>

Increased MicroRNA-1 and MicroRNA-133a Levels in Serum of Patients With Cardiovascular Disease Indicate Myocardial Damage

Yasuhide Kuwabara, MD; Koh Oto, MD, PhD; Takahiro Horie, MD, PhD; Hitoh Nishi, MD, PhD; Kazuya Nagao, MD, PhD; Minako Kinoshita, MD, PhD; Shin Watanabe, MD, PhD; Osamu Baba, MD; Yoji Kojima, MD, PhD; Satoshi Shizuta, MD; Masao Imai, MD; Toshihiro Tamura, MD; Toru Kita, MD, PhD; Takeshi Kimura, MD, PhD

Figure 1. MicroRNA (miR)-1 and miR-133a levels increased in the serum of patients with acute coronary syndrome (ACS). A and B, Expression levels of miR-1 (A) and miR-133a (B) in the serum of patients with non-ACS versus ACS. *P<0.0005, **P<0.0001. The levels of circulating miRNAs decreased over time in the serum of patients with ACS. C and D, miR-1 (C) and miR-133a (D) expression levels are shown according to the time of blood sampling after onset. Dots with daggers indicate samples without elevation of creatine phosphokinase or cardiac troponin T. Horizontal lines indicate the median.
MicroRNA in Acute Coronary Syndrome

Figure 2. miR-1 and miR-133a levels increased in the serum of patients with a variety of cardiovascular diseases. A and B, Expression levels of miR-1 (A) and miR-133a (B) in the serum of patients with cardiovascular diseases. NSTEMI indicates non-ST-segment elevation-myocardial infarction; STEMI, ST-segment elevation-myocardial infarction; PAD, peripheral artery disease; and AAA, abdominal aortic aneurysm. Horizontal lines indicate the median.
MicroRNA in Acute Coronary Syndrome

Figure 3. Circulating miR-133a is more sensitive and suitable as a biomarker for acute coronary syndrome (ACS) compared with circulating miR-1. A and B, Receiver operating characteristic analyses of miR-1 (A) and miR-133a (B) for diagnosis of ACS. C and D, Correlations between circulating miR-1 (C) or miR-133a (D) and cardiac troponin T (cTnT) in serum (n=92). AUC indicates area under the curve.

Circulation: Cardiovascular Genetics. 2011;4:446–454
MicroRNA in Acute Coronary Syndrome

Diagnostic potential of circulating miR-499-5p in elderly patients with acute non ST-elevation myocardial infarction

Fabiola Olivieri a,c,* , Roberto Antonicelli d, Maria Lorenzi c, Yuri D’Alessandra h, Raffaella Lazzarini e, Gabriele Santini b, Liana Spazzafumo f, Rosamaria Lisa d, Lucia La Sala a, Roberta Galeazzi c, Rina Recchioni c, Roberto Testa g, Giulio Pompilio h,i , Maurizio C. Capogrossi l, Antonio Domenico Procopio a,c

MicroRNA in cardiomyopathy

Identification of micro-RNA networks in end-stage heart failure because of dilated cardiomyopathy

Xiaoming Zhu a, b, Hongjiang Wang a, b, Fan Liu b, d, Li Chen c, d, Weijia Luo b, Pixiong Su b, Weiming Li a, Liping Yu c, Xinchun Yang a, *, Jun Cai a, *

Fig. 1 Schematic depiction of the experimental design and flowchart of the steps performed in this study.

MicroRNA in cardiomyopathy

Fig. 9 Quantitative RT-PCR for significantly altered miRNAs in DCM and control samples. Blue bars represent the DCM group, and green bars represent the control group (*P < 0.05).
A signature of circulating microRNAs differentiates takotsubo cardiomyopathy from acute myocardial infarction

Table 1  Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>TTC (n = 36)</th>
<th>STEMI (n = 27)</th>
<th>Healthy (n = 28)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (± SD)</td>
<td>68.7 (± 12.0)</td>
<td>67.1 (± 7.4)</td>
<td>63.7 (± 5.1)</td>
<td>0.093</td>
</tr>
<tr>
<td>Female gender, n (%)</td>
<td>33 (92)</td>
<td>23 (85)</td>
<td>24 (86)</td>
<td>0.680</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4 (± 4.5)</td>
<td>27.6 (± 6.9)</td>
<td>25.1 (± 4.5)</td>
<td>0.020</td>
</tr>
<tr>
<td>LVEF (%), mean (± SD)</td>
<td>40.7 (± 10.3)</td>
<td>47.1 (± 11.3)</td>
<td>60.5 (± 3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension, n (%)</td>
<td>20 (56)</td>
<td>17 (63)</td>
<td>15 (54)</td>
<td>0.648</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>7 (19)</td>
<td>12 (44)</td>
<td>10 (36)</td>
<td>0.077</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>10 (28)</td>
<td>9 (33)</td>
<td>1 (4)</td>
<td>0.007</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (8)</td>
<td>3 (11)</td>
<td>5 (18)</td>
<td>0.519</td>
</tr>
<tr>
<td>Positive family history, n (%)</td>
<td>11 (31)</td>
<td>10 (37)</td>
<td>6 (21)</td>
<td>0.292</td>
</tr>
<tr>
<td>Cardiovascular history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-obstructive CAD²</td>
<td>6 (17)</td>
<td>0</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obstructive CAD², n (%)</td>
<td>3 (8)</td>
<td>27 (100)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Re-animation, n (%)</td>
<td>3 (8)</td>
<td>6 (22)</td>
<td>0</td>
<td>0.155</td>
</tr>
<tr>
<td>Laboratory values on admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP (ng/L), median (IQR)</td>
<td>449.0 (172.0–3065.0)</td>
<td>396.4 (157.9–1479.8)</td>
<td>145.0 (115.0–194.0)</td>
<td>0.110</td>
</tr>
<tr>
<td>Troponin (μg/L), median (IQR)</td>
<td>0.3 (0.1–0.7)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>hs-Troponin (ng/mL), median (IQR)</td>
<td>257.0 (74.5–267.0)</td>
<td>350.0 (43.3–1545.0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CK (U/L), median (IQR)</td>
<td>1490 (105.0–197.3)</td>
<td>491.0 (208.3–1582.0)</td>
<td>107.0 (73.5–157.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>CKMB (U/L), median (IQR)</td>
<td>14.0 (6.83–26.0)</td>
<td>132.0 (19.5–255.0)</td>
<td>14.0 (11.3–17.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication on admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-inhibitor or ARB, n (%)</td>
<td>11 (31)</td>
<td>4 (15)</td>
<td>13 (46)</td>
<td>0.071</td>
</tr>
<tr>
<td>Beta-blocker, n (%)</td>
<td>11 (31)</td>
<td>1 (4)</td>
<td>11 (39)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Defined as a myocardial infarction in direct blood relatives of the patient (male <55 years, female <65 years).
²Atherosclerosis and lesions <50%.
*At least one stenosis of ≥50%.
ACE: angiotensin-converting enzyme; ARB: angiotensin-receptor blocker; BMI: body mass index; CAD: myocardial infarction; CK: creatine kinase; CKMB: creatine kinase MB-isoenzyme; LVEF: left ventricle ejection fraction; NT-proBNP: N-terminal pro-hormone brain natriuretic peptide; STEMI: ST-segment elevation acute myocardial infarction; TTC: takotsubo cardiomyopathy; IQR: inter-quartile range.
Figure 2. Comparative analysis of expression levels for selected microRNA candidates for takotsubo cardiomyopathy, ST-segment elevation acute myocardial infarction, and healthy controls; \( *P < 0.05, **P < 0.001, ***P < 0.0001 \).
MicroRNA in Arrhythmias

MicroRNA in Arrhythmias

Analysis of the microRNA signature in left atrium from patients with valvular heart disease reveals their implications in atrial fibrillation

Rosa Doñate Puertas1, Audrey Jalabert2, Emmanuelle Meugnier2, Vanessa Euthine7, Philippe Chevalier15++, Sophie Rome15++

A mRNA profiling

B mRNA profiling

Atrial Fibrillation

Normal

Normal sinus rhythm
Sinus (SA) node

Normal electrical pathways

Abnormal electrical pathways

Left atrium

LA/PV/LA junction

5,868

1,233

549

Up-regulated mRNAs

Targets of the down-regulated microRNA

mRNA/mRNA-Negative Regulation Pairs

3,313

687

379

Down-regulated mRNAs

mRNA/mRNA-Negative Regulation Pairs

Proliferation/microtubule dynamic/ion flux

MAP kinase signal transduction pathway

Regulation of mRNA splicing

Cell adhesion/Cell contact

(map kinase signaling transduction pathway)

(TGF)-β factor signaling pathway

Insulin-like growth factor 1 (IGF-1) axis

mRNA machinery

Co-expression analysis

(results in Figs 4 and 5)

mRNA target gene functional analysis

(results in Figs 4 and 5)

mRNA target gene predictions

miRNA/miRNA expression networks altered

PLOSONE | https://doi.org/10.1371/journal.pone.0196666
MicroRNA in Arrhythmias

Atrial Fibrillation

Our way to microRNA research
Our way to microRNA research

Block in Artery

Acute myocardial infarction

Muscle Damage

Necrosis
Loss of viable myocardium
Current Therapies

Percutaneous coronary intervention & Bypass Surgery

Medication

- Aspirin
- Betablockers
- ACE-Inhibitors
- Statins
- P2Y₁₂-Inhibitors
Interventional Therapies

Replace and Regeneration

Medication

- Aspirin
- Betablockers
- ACE-Inhibitors
- Statins
- P2Y₁₂-Inhibitors

Restore perfusion

Reduce Ventricular Remodeling and the risk of recurrent MI

http://www.salk.at/544.html
http://www.nycsurgical.net/
http://www.hopkinsmedicine.org
The Breakthrough?
Clinical Stem Cell Therapy in Myocardial Infarction

Circulation. 2003; 107: 929-934
Cells or Proteins?

https://en.wikipedia.org/wiki/White_blood_cell
www.goldbio.com
Mechanisms Behind (Stem) Cell Therapy

MSC paracrine action/mechanisms in heart regeneration... - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/280999752_fig2_Figure-1-MSC-paracrine-action-mechanisms-in-heart-regeneration-Soluble-factors-released [accessed Jan 24, 2016]

Circulation Research November 21, 2008 vol. 103 no. 11 1300-1308
Paracrine Effects

Dr. Stefano Di Santo, Bern 2009


humrep.oxfordjournals.org
A new approach

Production of rATG

Human Jurkat T Cells

Production of Antibodies against T cell antigens

- e.g., CD2, CD3, CD4, CD8, CD11a, CD18, CD29, CD44, CD45,

Activation and Induction of apoptosis

Immunisation

Immunoadsorption

Purification Distribution

Paracelsus Medizinische Privatuniversität
Experimental Design

Cytokine Secretion

- IL-2
- TNF-alpha
- Interleukin-1beta
- Interleukin-6

pro-angiogenic CXC and CC Chemokines

- Interleukin-8
- GRO-alpha
- ENA-78

In vitro Experiments

Flow Cytometry

Induction of Apoptosis

RT-PCR

ELISA

Induction of Cytokine/Chemokine Synthesis
Results

ELISA Assays

Pro-angiogenic Chemokines

- IL-2 pg/ml
- IL-8 pg/ml
- IL-6 pg/ml
- TNF-alpha pg/ml
- IL-10 pg/ml
- GRO-alpha pg/ml
- ENA-78 pg/ml
- MCP-1 pg/ml

n=4

control
100µg ATG
500µg ATG

Results

**in vivo Experiments**

**Scar Dimension 6 Weeks after Induction of MI**

Control

ATG

*neoangiogenesis in the peri-infarct area*

**Area of fibrosis after 6 weeks**

% of left ventricle

(control) vs. (ATG)

n=8-13 per group

Mechanism of Action

Apoptotic PBMC → Secretion of soluble factors → T Cell

Cardioprotective effects

Cardiac myocyte

Cytokine receptors

Growth factor receptors

RAS

PI-3K

ERK1/2

AKT

JNK

Bcl-2

BAG1

Inhibition of apoptosis

Cytoskeleton stabilization

Cardiac survival genes

Cytoplasm

Nucleus

Cardioprotection
Interventional Therapies

Paracrine Therapies
- Cardioprotection
- Neoangiogenesis
- Stem Cell Therapy
- Platelet aggregation
- Vasodilation
- Extracellular matrix/remodeling

Medication
- Aspirin
- Betablockers
- ACE-Inhibitors
- Statins
- P2Y$_{12}$-Inhibitors

Prevention of Heart Failure
Membrane Array Analysis

Control

CC and CXC Chemokines

Pro-angiogenic Factors

ATG

Interleukin-8
ENA-78
GRO-alpha
MCP-1
MIP-1, MIP-3
AMI
Small Animal Model

Intravenous injection of anti-CD3 antibodies
Mechanism of action? Exosomes?

Stem Cell Reports

Article

Exosomes as Critical Agents of Cardiac Regeneration Triggered by Cell Therapy

Ahmed Gamal-Eldin Ibrahim, Ke Cheng, and Eduardo Marbán.*

©Harvest Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

*Correspondence: eduardo.marban@chsu.org

https://dx.doi.org/10.1016/j.stemcr.2014.04.006

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FACS and ExoELISA
Exosome Quantification

Isotype
CD3
ATG

Exosomes (10^6)
Exosome Analysis
Next Generation Sequencing

Exosomes from cultures of peripheral blood mononuclear cells treated with:
- ATG
- anti-CD3
- control
Several good candidates have been identified.
microRNAs with mean TPM ≥ 10 and CV > 50% (n=29)

Analysis of rat myocardium after ischaemia vs. sham controls

microRNAs with average TPM ≥ 10 and CV > 50% (n=29)
Top miRNAs from the PBMC microvesicle project: expression profiles in heart tissue of ischemic rats
Several good candidates have been identified.
Administration of recombinant miR-30d in vivo

30 minutes ischaemia/reperfusion injury

miR-30 precursor
miR-30

- Apoptosis
- Autophagy
- Inflammation
- Oxidative Stress
- Ventricular remodelling

Target analysis

MicroRNAs in Myocarditis

MicroRNAs in Myocarditis

30 most variant microRNAs

Volcano Plot for EAM vs Co

The top 20 microRNA by p-value (unadjusted) are annotated in the plot.

Count Plot for Top 5 microRNAs by p-value for EAM vs Co
MicroRNAs in Myocarditis

Count Plot for Top 5 microRNAs by p-value for EAM vs Co

Note that the following plots show RPM values and not the count data used internally in edgeR after library normalization (TMM values).

Top 5 upregulated microRNAs by p-value

log10 RPM Value

microRNA

- miR-1-46b-3p
- miR-1-47-3p
- miR-21-5p
- miR-21-5p
- miR-223-3p

group Co EAM
MicroRNAs in Myocarditis

The diagram illustrates the role of microRNAs in myocarditis, highlighting cellular response, fibrosis, cytokines, viral replication, cardiac arrhythmias, connexins, and apoptosis. Key microRNAs and pathways include:

- *Cellular response*: miR-155, miR-21, miR-146b, miR-125b.
- *Fibrosis*: miR-148a, miR-590-3p, miR-125b, miR-146b.
- *Viral replication*: miR-221/222, miR-223, miR-203, miR-20b, ZFP-148, SPRED1.
- *Cardiac arrhythmias*: miR-203, miR-19b, Cx43.
- *Connexins*: miR-1.
- *Apoptosis*: SIRT1, FAS/FASL, miR-98.

The diagram shows the interplay between these microRNAs and their targets, contributing to the understanding of myocarditis pathogenesis.
MicroRNA in Cardiovascular Disease

Hypertrophy
- miR-22
- miR-212/132
- miR-199b
- miR-199a
- miR-206
- miR-154
- miR-410
- miR-495
- miR-541
- miR-133
- miR-1

Pten
FoxO3
Dyrk1a
Gak3b
Foxp1
Cdkn2b
Pten
NA
NA
Itprl2, Serc2a, calcineurin
Cdk6

Myocardial infarction
- miR-294
- miR-133
- miR-539
- miR-410
- miR-495
- miR-433
- miR-15
- miR-195
- miR-497
- miR-590-3p
- miR-199a-3p
- miR-133

Wee1
Snail1
Ptk2
Cited2
Cited2
Azin1, Jnk1
NA
NA
Bcl2, Map1lc3b
Homer1, Hopx, Clic5
Homer1, Hopx
Casp9

Contractility defects
- miR-27a
- miR-1
- miR-208a
- miR-22

Myh7
α-Mhc
Myh7, Myh7b
Purβ

Arrhythmias
- miR-1
- miR-133
- miR-17-92
- miR-206

Pp2a
Pp2a
Cx-43, Pten
Cx-43

Inherited cardiomyopathies

ACM

miR-217-5p
miR-708-5p
miR-130a
miR-130
miR-21-5p
miR-499-5p
miR-135b

NA
NA
NA
NA
NA
NA
NA

HCM

miR-204
miR-17-5p
miR-155
miR-10b
miR-23a
miR-139-5p
miR-1-3p

NA
NA
NA
NA
NA
NA
NA

DCM

miR-208b
miR-155
miR-15
miR-23a
miR-21
miR-148a
miR-27a

NA
NA
NA
NA
NA
NA
NA

LQTS

miR-134
miR-103a-1
miR-143
miR-3619
miR-1
miR-133a

hERG
hERG
hERG
hERG
NA
NA

Cells 2019, 8(7), 737
Conclusion

Multiple ways of application for microRNAs in Cardiology

Understanding of pathophysiology
Risk prediction
Diagnosis
Therapy

Basic science is making good progress

Large clinical trials are needed
Technology has to become more widely available
Costs have to come down
Thank you!

Special thanks to:
Dr. Bernhard Werny, Dr. Attila Kiss, Vera Paar, Prof. Uta C. Hoppe, Prof. Bruno Podesser, Dr. Matthias Hackl