MicroRNAs as therapeutic targets in Cardiac disease

ESC Summer school Nice, June 2015

Eva van Rooij
**Remodeling of the heart**

**Normal Heart**
- Exercise, Pregnancy

**Hypertension, MI and Neurohumoral Activation**
- Myocyte hypertrophy
- Apoptosis
- Fibrosis
- Metabolic shift
- Reduced cAMP generation
- Fetal gene activation (α → β - myosin switch)

**Physiologic Remodeling**

**Pathologic Remodeling**

**Heart Failure**

**Stress**
MicroRNA biogenesis & conservation

Fish miR-214
Chimpanzee miR-214
Rhesus Macaque miR-214
Rat miR-214
Opossum miR-214
Wild boar miR-214
Zebrafish miR-214
Xenopus miR-214
Cow miR-214
Human miR-214
Mouse miR-214
Lizard miR-214
Carp miR-214

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A microRNA signature of heart disease

van Rooij et al. PNAS 2006
miR-15 family is upregulated in human heart failure

“Ongoing loss of cardiac myocytes is responsible for the development and worsening of heart failure.”
Cardiac microRNA regulation during neonatal cell cycle arrest

Up-regulated

hsa-let-7d*
hsa-miR-15a*
hsa-miR-100
hsa-miR-15b*
hsa-miR-155
hsa-miR-152
hsa-miR-451
hsa-miR-125a-5p
hsa-miR-486-3p
hsa-let-7f
hsa-let-7c
hsa-let-7g
hsa-miR-139-5p
hsa-let-7e
hsa-let-7b
hsa-miR-155*
hsa-miR-146a
hsa-miR-30b
hsa-miR-148a
hsa-miR-159a-3p
hsa-miR-23a
hsa-miR-23b
hsa-miR-16*
hsa-miR-125b
hsa-miR-150
hsa-miR-486-5p
hsa-miR-151-5p
hsa-miR-499-5p
hsa-miR-574-3p
hsa-miR-197c
hsa-miR-130a
hsa-miR-30c
hsa-let-7d
hsa-miR-30a
hsa-miR-30e
mmu-miR-505
hsa-miR-24
hsa-miR-378
hsa-miR-27b
hsa-miR-99a
mmu-miR-805

Down-regulated

hsa-miR-103
hsa-miR-1275
hsa-miR-1277
mmu-miR-329
hsa-miR-503
mmu-miR-667
hsa-miR-941
hsa-miR-1469
hsa-miR-23a*
mmu-miR-224
mmu-miR-29b*
hsa-miR-1259
hsa-miR-20a
hsa-miR-433
hsa-miR-1268
mmu-miR-290-5p
hsa-miR-940
hsa-miR-329
mmu-miR-322*
hsa-miR-638
mmu-miR-483
mmu-miR-351
mmu-miR-629
hsa-miR-107
hsa-miR-371-5p
hsa-miR-208b
hsa-miR-220a
hsa-miR-498
mmu-miR-705
mmu-miR-762

p1 versus p10 hearts

Up-regulation of miR-15 family

Porrello et al. Circ Res 2011
miR-15 controls cell cycle and survival

**Cyclins:**
- Cyclin E1
- Cyclin D2
- Cyclin T2
- Cyclin M2
- Cyclin D1
- Cyclin D3

**Cell cycle / survival:**
- CDCA4
- CDC42
- BCL2L2
- CDC27
- Smad7
- E2F7
- Smad5
- PDCD4
- PBBP6
- CDC37L1
- CHEK1
- CDC25A
- CDC14B
- CDK5R1
- Smad3
- CAPRIN1
- CDC14A
- E2F3
- Tcl-1

**Targets**
- PDCD6IP
- CDC23
- G0S2
- CCNJL
- CDC25B
- CDCA7L
- CRKRS
- PAK7
- CDK6
- BCL2

**Stress signals**

**miR-15 family**

**Myocyte differentiation & survival**

Porrello et al. Circ Res 2011
MicroRNA expression in response to ischemic injury

Regulated miRNAs in borderzone of infarcted area

- Upregulated: 6, 11, 21
- Downregulated: 8, 15, 22

3 days post-MI
14 days post-MI

van Rooij et al. PNAS 2008
miR-29 regulates ECM

- Decrease in miR-29 leads to tissue fibrosis
- Increase in miR-29 results in plaque destabilization
Cardiac myosin switching

**Stress**
- **αMHC** - high ATPase activity
  - downregulated during heart disease
  - upregulated by thyroid hormone

**Hypothyroidism**
- **βMHC** - low ATPase activity
  - upregulated during heart disease
  - upregulated by hypothyroidism

**Slow skeletal muscle**
- **βMHC**
miR-208 is cardiac specific and co-expressed with \( \alpha \)MHC.

\[ \alpha \text{Myosin heavy chain} \]

![Graph showing expression levels of miR-208 and \( \alpha \)MHC across different tissues.](image)

van Rooij et al. Science 2007
Genetic deletion of miR-208 blocks stress-induced remodeling and $\beta$MHC expression
miR-208 targets Thrap1 / MED13

MED13 (Thrap1)  

Arg finger  
NLS  
Nuclear receptor binding motifs  
Forkhead-N Like domain  

miR-208a/b  
miR-499  

431-439  
531-533  
757  
4234  

MED13 3' UTR  

α-THRAP1

WT  
KO
miR-208 controls a network of transcriptional repressors

myosins

MyomiRs

MED13

TR

Purβ

Sox6

Sp3

HP-1β

Class II HDAC

MEF2

Stress-responsive genes

cardiac fibrosis and hypertrophy

van Rooij et al. Dev Cell 2009
Basics of microRNA biology

- MiRNAs are conserved across species
- MiRNAs are regulated during disease
- MiRNAs are more important under stress conditions, i.e. during disease
- The genome can contain multiple copies of a microRNA and they often target multiple genes involved in a similar cellular process or signaling pathway
- Intronic miRNAs often influence the function of the host gene
- Presence of multiple binding sites within an UTR can correlate to the relevance of the miRNA/mRNA interaction
- The level of gene regulation is moderate, but the combined influence of these relatively small changes can have a profound downstream effects
MicroRNA function in cardiac disease

van Rooij et al., PNAS, 2006, 2008; Science, 2007; JCI, 2007; Dev Cell, 2009; Wang et al., Dev Cell, 2008; Liu et al., Genes & Dev, 2008; Xin et al., Genes & Dev, 2009; Williams et al., Science, 2009
MicroRNA modulation
### Bringing MicroRNA Discoveries to the Clinic

<table>
<thead>
<tr>
<th>Company</th>
<th>Location</th>
<th>Disease Focus</th>
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<tr>
<td>Asuragen</td>
<td>Austin, Texas</td>
<td>Cancer diagnostics</td>
<td>2006</td>
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<tr>
<td>Cogen Pharmaceuticals</td>
<td>Philadelphia</td>
<td>Cancer</td>
<td>2004</td>
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<tr>
<td>Mitagen Therapeutics</td>
<td>Boulder, Colorado</td>
<td>Cardiovascular and muscle diseases</td>
<td>2007</td>
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<tr>
<td>Regulus Therapeutics</td>
<td>Carlsbad, California</td>
<td>Viral diseases, cancer</td>
<td>2007</td>
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<tr>
<td>Rosetta Genomics</td>
<td>Rehovot, Israel, and Jersey City, New Jersey</td>
<td>Cancer</td>
<td>2000</td>
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**Science, 28 March 2008**

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**October 2011**

**Servier and Miragen Sign $352 Million Partnership Agreement for the Research, Development and Commercialization of MicroRNA-targeting Drugs for Cardiovascular Disease**
AntimiR chemistries

van Rooij and Olson. Nature Rev Drug Discovery
miR-15 family upregulated in response to ischemic injury in pigs
miR-15 family inhibition using antimiRs

Experimental set-up:
- IV injection C57Bl6
- Doses ranging from 0.033 mg/kg to 33 mg/kg
- Collected tissues 7 days after injection
- Determined knockdown by realtime PCR analysis
miR-15 family inhibition induces target derepression in cardiomyocytes
AntimiR-15 reduces infarct size in response to ischemic injury

Hullinger et al. Circ Res 2011
miR-15 inhibition reduces remodeling and improves cardiac function in response to ischemic damage.
AntimiR-208a

miR-208

Montgomery et al. Circ 2012
Dahl Salt-Sensitive rat model

Dahl Salt-Sensitive Model is a high-quality rodent model for diastolic heart failure

High salt diet causes continuous increase in blood pressure which leads to remodeling of the heart

- hypertrophy
- fibrosis
- increase hypertrophic markers
- switch from $\alpha$MHC to $\beta$MHC

Stiffening of the ventricle diminishes elasticity, relaxation of the LV

<table>
<thead>
<tr>
<th>8</th>
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<td>echo &amp; antimIR</td>
<td>echo</td>
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</tr>
</tbody>
</table>
Therapeutic Inhibition of miR-208a Improves Cardiac Function and Survival During Heart Failure

Rusty L. Montgomery, PhD; Thomas G. Hullinger, PhD; Hillary M. Semus, MS; Brent A. Dickinson, BS; Anita G. Seto, PhD; Joshua M. Lynch, BS; Christianna Stack, MS; Paul A. Latimer, BS; Eric N. Olson, PhD; Eva van Rooij, PhD

Montgomery et al. Circ 2012
AntimiR-208 improves survival during heart failure

![Survival graph showing the percent survival over weeks post 4% NaCl diet. The graph compares different treatment groups: LS/Saline, HS/Saline, HS/25 mg/kg antimiR-208a, and HS/Control. The graph highlights the improvement in survival with AntimiR-208 treatment.]
MicroRNA modulation

The diagram illustrates the process of microRNA (miRNA) modulation, showing the steps from pri-miRNA to mature miRNA (pre-miRNA to mature miRNA) and the roles of Drosha and Exportin 5. It also highlights the effects of miRNA on gene expression through translational repression and miRNA degradation, resulting in decreased or increased protein expression.

Key steps include:
- **Add miRNA Mimics**
- **Add miRNA Inhibitors**
- **mRNA**
- **ORF**
- **AAA**
- **Ribosome**
MicroRNA mimicry in multiple tissues

Montgomery et al. EMBO Mol Med 2014
The stability of a microRNA mimic is tissue dependent.

100 mg/kg iv
miR-29 mimicry blocks pulmonary fibrosis
miR-29 mimicry reverses signs of pulmonary fibrosis
pH sensitive Upy hydrogel for cardiac delivery of novel therapeutics

Dankers, TU/e
In vivo delivery of Upy hydrogel in porcine myocardial infarction model

Towards other drugs and possibly other formulations

Dankers, TU/e

TU/e and UMC Utrecht
pH sensitive Upy hydrogel for cardiac delivery of microRNA therapeutics
MicroRNA therapeutics

- AntimiRs can induce longlasting, potent and specific inhibition of a microRNA
- AntimiRs can be used to target cardiac microRNAs, but preferentially deliver to the kidney and liver – *what are the implications for chronic indications?*
- AntimiRs can be delivered subcutaneously (but do not cross the GI tract) – *will an injectable be attractive enough?*
- MicroRNA mimic can increase a microRNA in vivo
Efficacy studies using antimiRs in rodents
Species dependent target regulation

Target regulation in mice

Target regulation in humans

microRNA

phenotype
MicroRNA-92a Controls Angiogenesis and Functional Recovery of Ischemic Tissues in Mice

Angelika Bonauer,1 Guillaume Carmona,1 Masayoshi Iwasaki,1 Marina Mione,2 Masamichi Koyanagi,1 Ariane Fischer,1 Jana Burchfield,1 Henrik Fox,1,3 Carmen Doebele,1 Kisho Ohtani,1 Emmanouil Chavakis,1,3 Michael Potente,1,3 Marc Tjwa,4 Carmen Urbich,1 Andreas M. Zeiher,3 Stefanie Dimmeler1*

Slice 2

Slice 3

Non-ischemic regions

Ischemic regions
miR-92a inhibition reduces infarct size and enhances cardiac function in a porcine IR model

Hinkel et al. Circ 2013
Treatment of HCV Infection by Targeting MicroRNA

**Figure 2. Change from Baseline in HCV RNA Levels.**

Shown are the mean changes in HCV RNA levels from baseline for patients receiving 3 mg, 5 mg, or 7 mg of miravirsen per kilogram of body weight, as compared with placebo. Miravirsen was administered in five weekly subcutaneous injections during the first 29 days of the study (gray shading). The dashed line indicates no change from baseline. The HCV RNA levels during the use of pegylated interferon and ribavirin in some patients were not included in this analysis.

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Prof. Olson, UTSW

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Van Oudenaarden Group

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