MicroRNAs in cardiomyopathy

서울대학병원 순환기 내과

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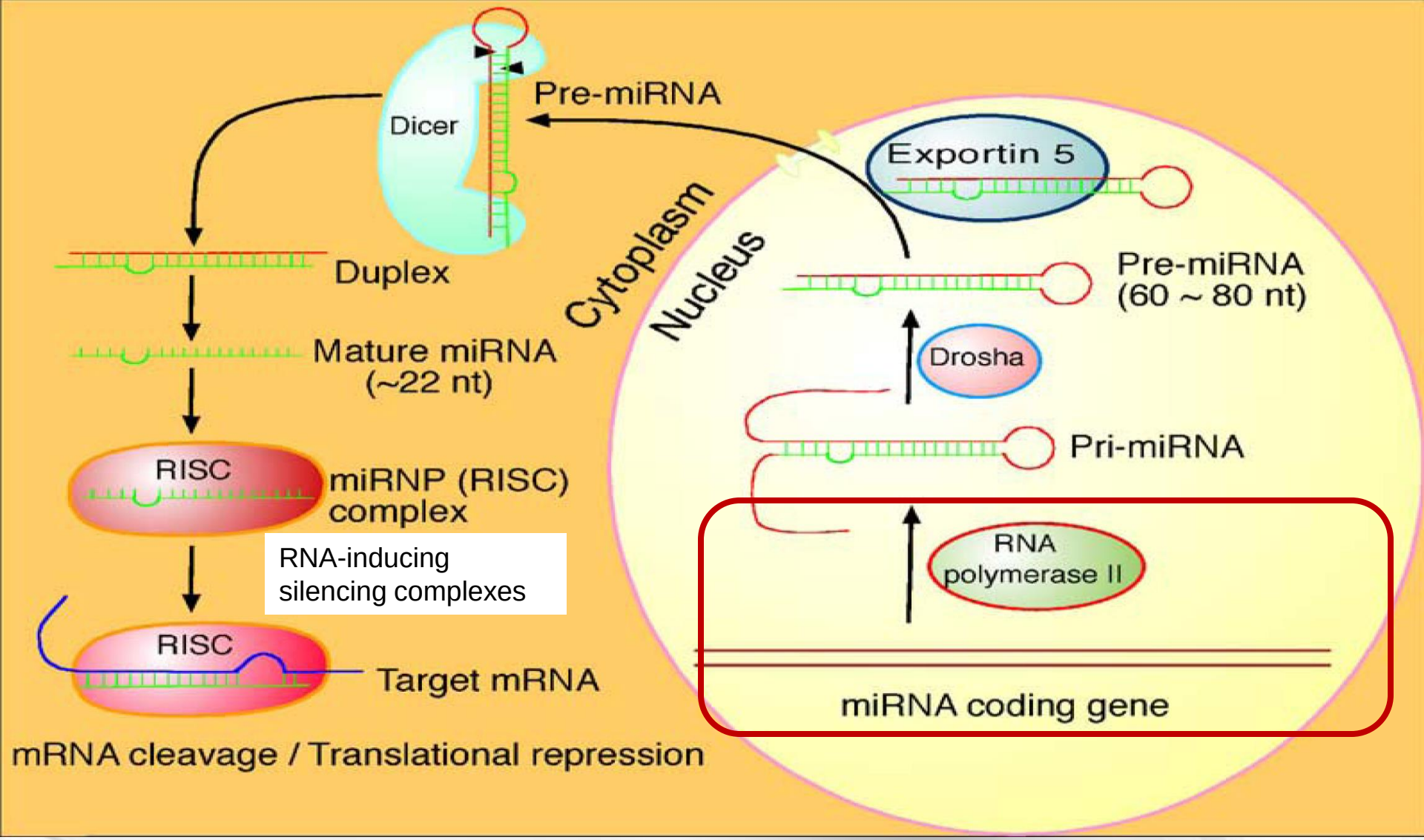
2013. 4. 20.

Contents

Introduction of microRNA

- Short (19-24 nucleotides) non-coding RNAs
- The first described microRNA, lin-4 was cloned and characterised from C. elegans (1993)
- Primarily functions as translational repressors by binding to complementary target sequences in the 3’ UTR (untranslated region) of mRNA.
- Between 60% of all human genes are a target for microRNA regulation (Firedman et al, 2009).
- A single target gene regulated by multiple microRNAs, a single microRNA regulates multiple genes.

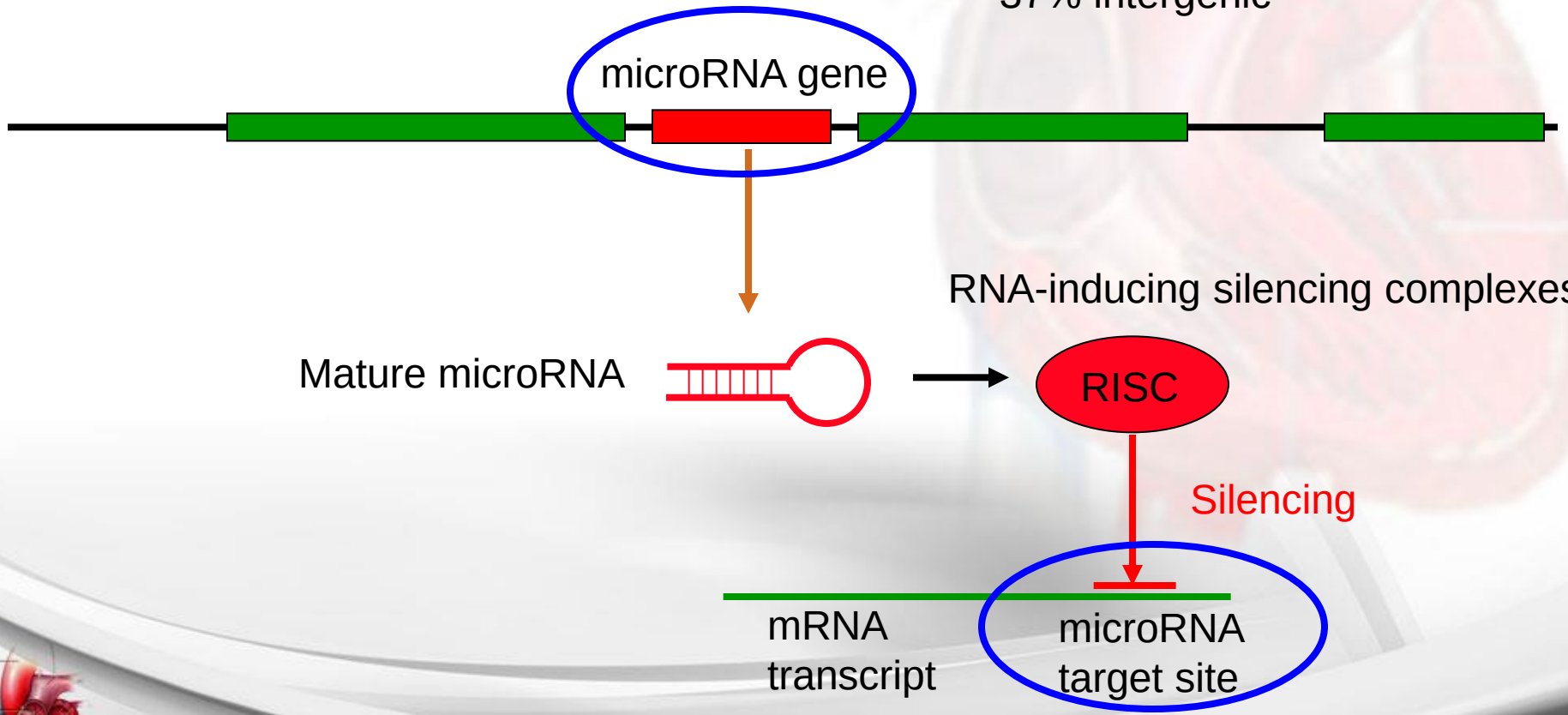


- **MicroRNAs are transcribed in a RNA Polymerase II-dependent manner as large polyadenylated pri-microRNAs.**
- **RNAPII catalyzes the transcription of DNA to synthesize precursors of mRNA and most snRNA and microRNA**

Yang CGFR 16:397, 2005

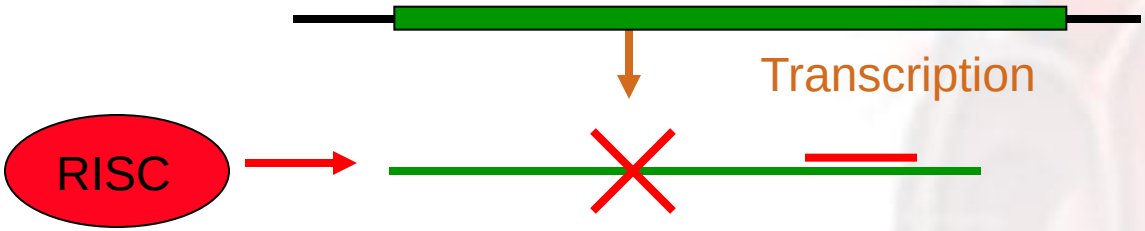
microRNA is mainly negative regulator of mRNA translation

Location of microRNA
- 40% introns of coding RNA
- 10% introns of noncoding RNA
- 13% exons of noncoding RNA
- 37% intergenic

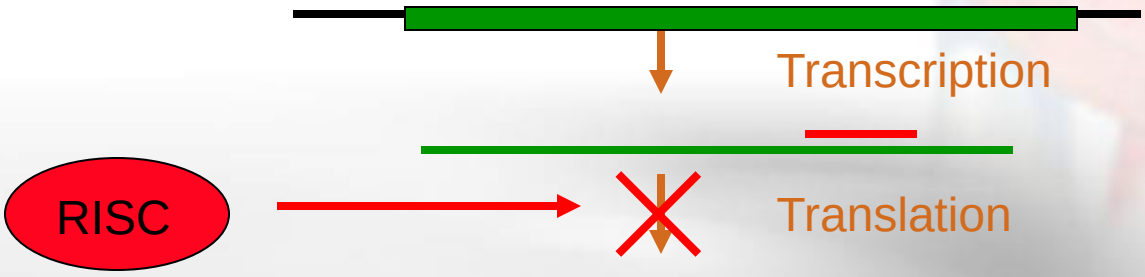


Mechanisms for microRNA regulation

- Post-transcriptional degradation of target mRNA transcript
  - microRNA triggers the destruction of target



- Translational repression
  - microRNA prevents translation to protein



TargetScanHuman
Prediction of microRNA targets
Release 6.0: November 2011

- Search for predicted microRNA targets in mammals (/worm/fly) 3’ UTRs.
- Find conserved 8mer and 7mer sites that match the seed region of each miRNA.
- Predictions are ranked based on the predicted efficacy of targeting as calculated using the context+ scores of the sites

TargetScanHuman

Prediction of microRNA targets

Release 6.0: November 2011

Search for predicted microRNA targets in mammals

[Go to TargetScanMouse]
[Go to TargetScanWorm]
[Go to TargetScanFly]

1. Select a species Human

AND

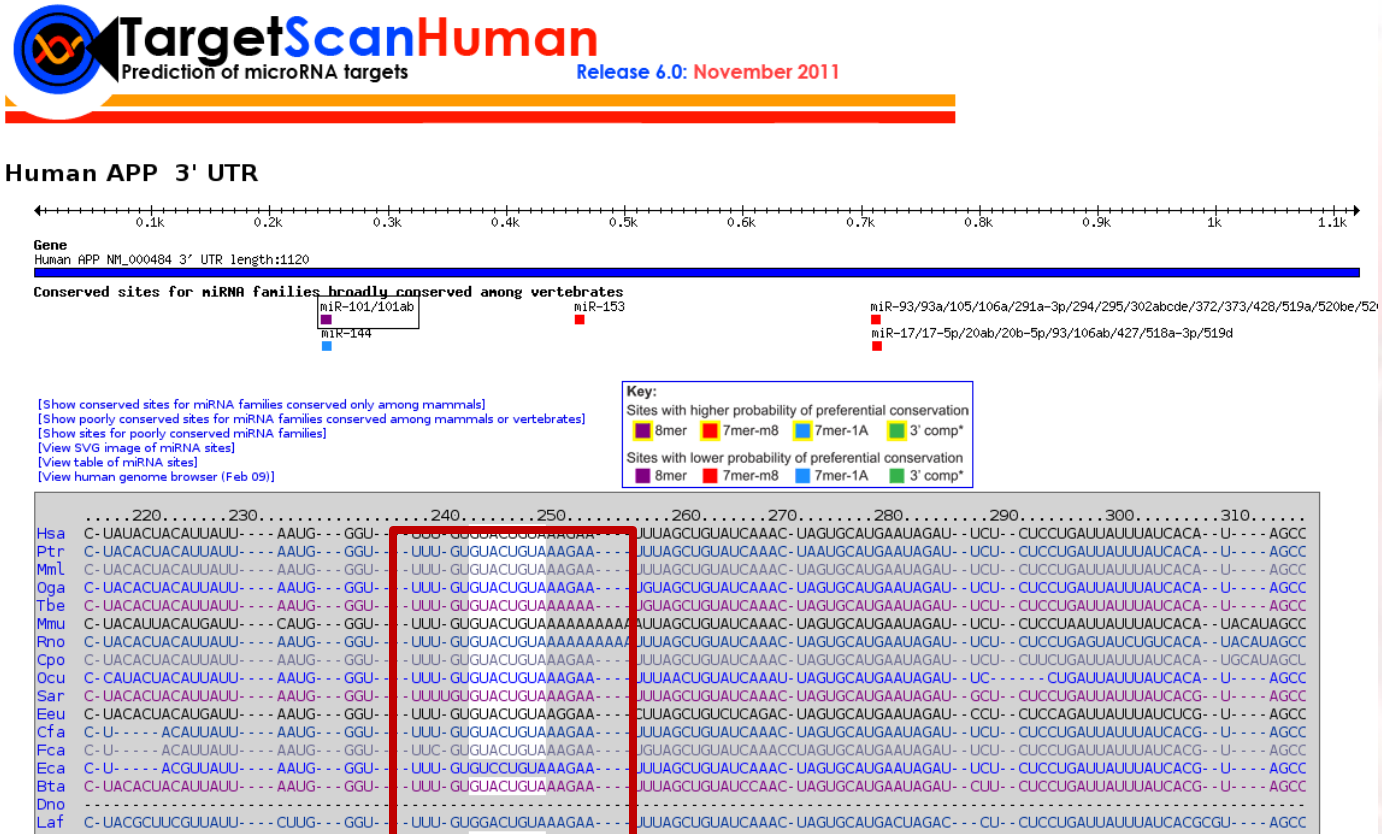
2. Enter a human Entrez Gene symbol (e.g. "LIN28A") app

AND/OR

3. Do one of the following:

- Select a broadly conserved* microRNA family: Broadly conserved microRNA families
- Select a conserved* microRNA family: Conserved microRNA families
- Select a poorly conserved microRNA family: Poorly conserved microRNA families Note that these families also include small RNAs that have be
- Enter a microRNA name (e.g. "mmu-miR-1")

Submit Reset



- **Aggregate P_{CT}** : identifying targeting interactions not only likely to be **effective** but also those that are more likely to be **consequential** for the animal.
- **Context scores**: predictive for all types of interactions, including those of miRNAs that are not highly conserved.

Conserved

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site-type contribution	3' pairing contribution	local AU contribution	position contribution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length	P_{CT}
Position 243-250 of APP 3' UTR	5' ...AUUAAUGGGUUUUGUGUACUGUA...	8mer	-0.247	-0.008	-0.062	-0.053	0.011	0.007	-0.35	96	1.619	0.66
hsa-miR-101	3' AAGUCAAUAGUGUCAUGACAU											

Context+ score and features that contribute to the context+ score are evaluated as in Garcia et al., 2011.
Conserved branch lengths and P_{CT} are evaluated as in Friedman et al., 2008.

Poorly conserved

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site-type contribution	3' pairing contribution	local AU contribution	position contribution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length	P_{CT}
Position 532-538 of APP 3' UTR	5' ...UCCAUGACUGCAUUUUACUGUAC...	7mer-1A	-0.074	0.004	-0.005	0.008	0.006	0.015	-0.05	32	0.859	< 0.1
hsa-miR-101	3' AAGUCAAUAGUGUCAUGACAU											

TargetScanHuman

Prediction of microRNA targets

Release 6.0: November 2011

Search for predicted microRNA targets in mammals

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- Enter a microRNA name (e.g. "mmu-miR-1")

Submit Reset

Mir 31 - broadly conserved* microRNA

Human | miR-31

368 conserved targets, with a total of **394** conserved sites and **153** poorly conserved sites.

Table sorted by total context score [Sort table by aggregate P_{CT}]

Genes with only poorly conserved sites are not shown [View top predicted targets, irrespective of site conservation]

The table shows at most one transcript per gene, selected for having the highest aggregate P_{CT} (or the one with the longest 3' UTR, in case of a tie). [Show all transcripts]

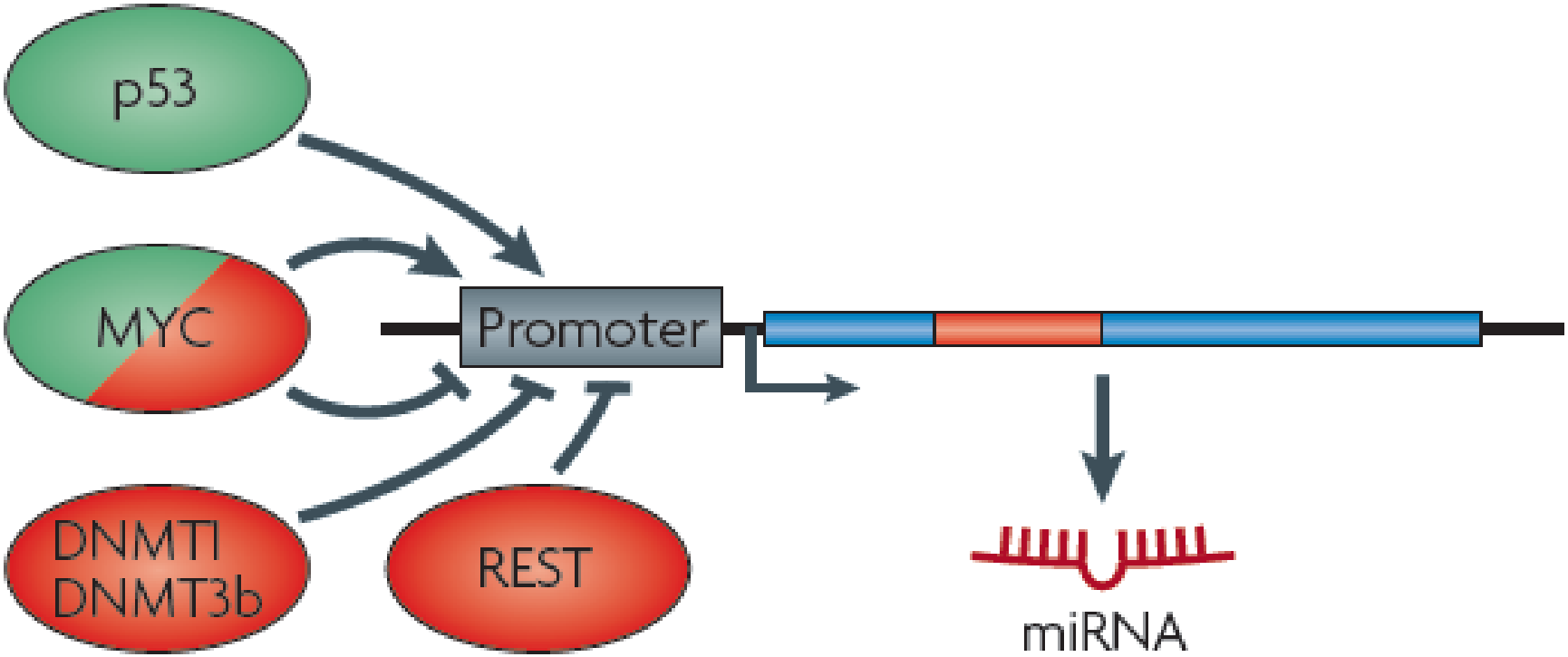
Target gene	Representative transcript	Gene name	Conserved sites				Poorly conserved sites				Repre-sentative miRNA	Total context+ score	Aggregate P_{CT}	Previous TargetScan publication(s)	Links to sites in UTRs
			total	8mer	7mer-m8	7mer-1A	total	8mer	7mer-m8	7mer-1A					
RSBN1	NM_018364	round spermatid basic protein 1	2	1	1	0	3	0	2	1	hsa-miR-31	-0.97	0.53	2007, 2009	Sites in UTR
ARHGEF2	NM_001162383	Rho/Rac guanine nucleotide exchange factor (GEF) 2	1	0	0	1	2	1	1	0	hsa-miR-31	-0.74	0.17		Sites in UTR
IDE	NM_001165946	insulin-degrading enzyme	2	1	1	0	1	0	1	0	hsa-miR-31	-0.69	0.41		Sites in UTR
NR5A2	NM_003822	nuclear receptor subfamily 5, group A, member 2	2	1	1	0	1	0	0	1	hsa-miR-31	-0.67	0.62	2007, 2009	Sites in UTR
SH2D1A	NM_001114937	SH2 domain containing 1A	2	1	1	0	0	0	0	0	hsa-miR-31	-0.65	0.58	2007, 2009	Sites in UTR
ZNF512	NM_032434	zinc finger protein 512	2	0	2	0	1	0	1	0	hsa-miR-31	-0.65	0.50		Sites in UTR
PRKCE	NM_005400	protein kinase C, epsilon	1	1	0	0	2	0	1	1	hsa-miR-31	-0.64	0.17	2009	Sites in UTR
PIK3C2A	NM_002645	phosphoinositide-3-kinase, class 2, alpha polypeptide	2	1	1	0	0	0	0	0	hsa-miR-31	-0.59	0.43	2009	Sites in UTR
PEX5	NM_000319	peroxisomal biogenesis factor 5	1	1	0	0	1	0	1	0	hsa-miR-31	-0.56	0.34	2007, 2009	Sites in UTR
SATB2	NM_001172509	SATB homeobox 2	2	1	1	0	0	0	0	0	hsa-miR-31	-0.56	0.56	2007, 2009	Sites in UTR
AKAP7	NM_004842	A kinase (PRKA) anchor protein 7	2	1	0	1	1	0	0	1	hsa-miR-31	-0.55	0.39	2007	Sites in UTR
TSGA10	NM_025244	testis specific, 10	1	1	0	0	0	0	0	0	hsa-miR-31	-0.54	0.34		Sites in UTR
RHOBTB1	NM_001242359	Rho-related BTB domain containing 1	1	1	0	0	1	0	0	1	hsa-miR-31	-0.54	0.21	2007, 2009	Sites in UTR
OAS2	NM_016817	2'-5'-oligoadenylate synthetase 2, 69/71kDa	1	1	0	0	2	0	1	1	hsa-miR-31	-0.53	0.36		Sites in UTR
SEPHS1	NM_001195602	selenophosphate synthetase 1	1	0	1	0	1	1	0	0	hsa-miR-31	-0.51	0.21	2007, 2009	Sites in UTR
DUSP7	NM_001947	dual specificity phosphatase 7	1	1	0	0	1	0	1	0	hsa-miR-31	-0.50	0.59		Sites in UTR
PPP1R9A	NM_001166160	protein phosphatase 1, regulatory (inhibitor) subunit 9A	1	1	0	0	0	0	0	0	hsa-miR-31	-0.50	0.21	2007, 2009	Sites in UTR
RNF144B	NM_182757	ring finger protein 144B	1	0	0	1	1	1	0	0	hsa-miR-31	-0.48	0.17	2007	Sites in UTR
KANK1	NM_015158	KN motif and ankyrin repeat domains 1	1	1	0	0	0	0	0	0	hsa-miR-31	-0.48	0.53	2007, 2009	Sites in UTR
SLC1A2	NM_001195728	solute carrier family 1 (glial high affinity glutamate transporter), member 2	2	2	0	0	2	0	1	1	hsa-miR-31	-0.48	0.54	2007, 2009	Sites in UTR
KIAA0889	NM_080627	KIAA0889	1	1	0	0	3	0	1	2	hsa-miR-31	-0.48	0.48		Sites in UTR
DCBLD2	NM_080927	discoidin, CUB and LCCL domain containing 2	1	1	0	0	1	0	1	0	hsa-miR-31	-0.48	0.40	2007, 2009	Sites in UTR
STARD13	NM_052851	StAR-related lipid transfer (START) domain containing 13	1	1	0	0	0	0	0	0	hsa-miR-31	-0.47	0.17	2009	Sites in UTR
HIF1AN	NM_017902	hypoxia inducible factor 1, alpha subunit inhibitor	1	1	0	0	3	0	2	1	hsa-miR-31	-0.47	0.67	2007, 2009	Sites in UTR
SLC6A6	NM_001134367	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	1	1	0	0	1	0	1	0	hsa-miR-31	-0.47	0.34	2007, 2009	Sites in UTR
ADCY6	NM_015270	adenylate cyclase 6	1	1	0	0	2	0	1	1	hsa-miR-31	-0.47	0.50	2009	Sites in UTR
SLC35A2	NM_005660	solute carrier family 35 (UDP-galactose transporter), member A2	1	0	1	0	1	0	1	0	hsa-miR-31	-0.46	0.21		Sites in UTR
TMPRSS11F	NM_207407	transmembrane protease, serine 11F	1	1	0	0	0	0	0	0	hsa-miR-31	-0.46	0.27	2009	Sites in UTR
RET	NM_020630	ret proto-oncogene	1	1	0	0	0	0	0	0	hsa-miR-31	-0.45	0.58		Sites in UTR

* conserved across most vertebrates, usually to zebrafish

What are the regulating mechanisms of microRNA?

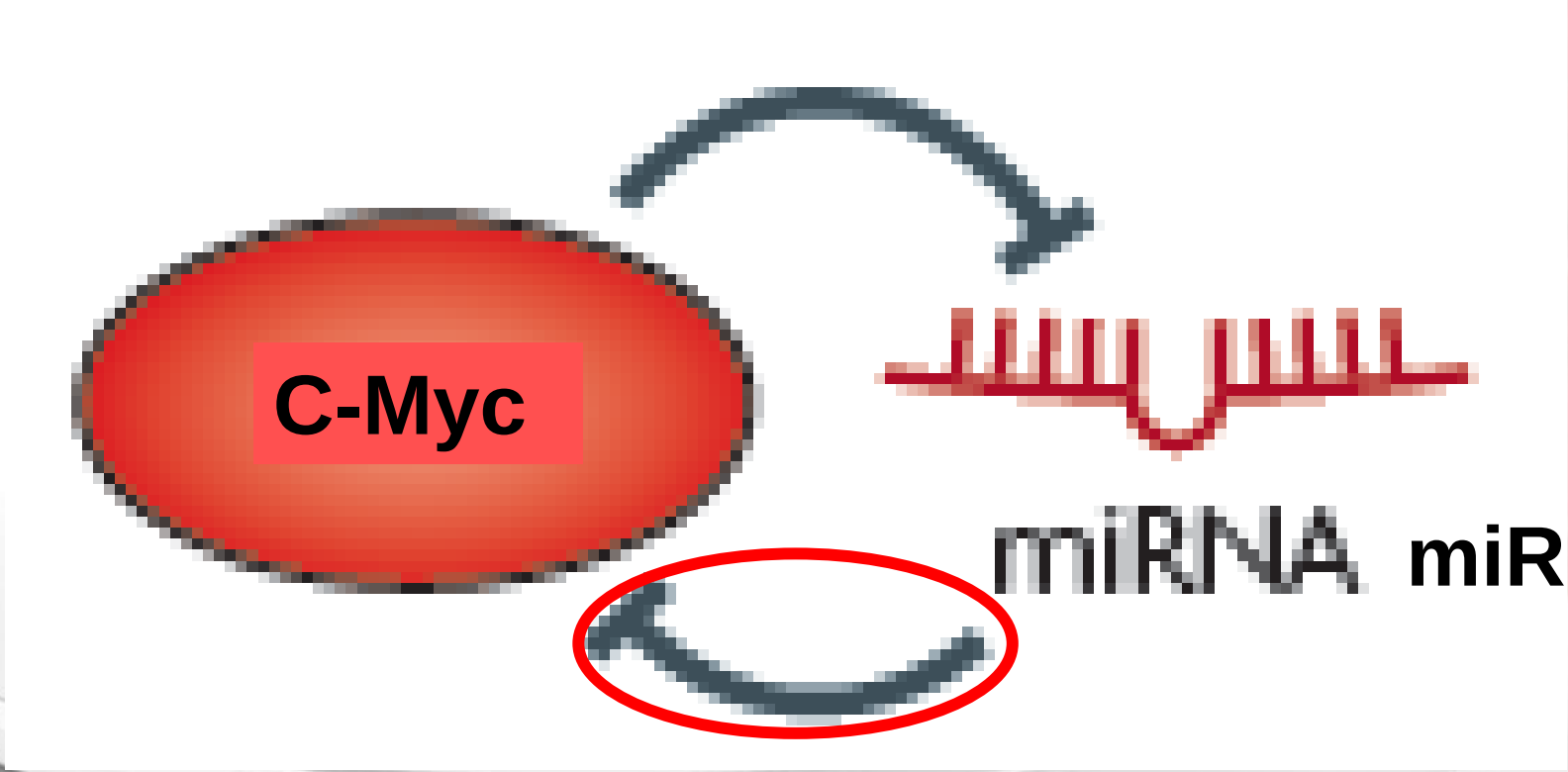
- Regulation of microRNA gene transcription
  - Activators / Repressors
  - Regulatory networks
- Regulation of microRNA processing
  - DROSHA, DICER, binding proteins
- Regulation of microRNA function
  - AGO, GW182

Transcriptional regulation of microRNA



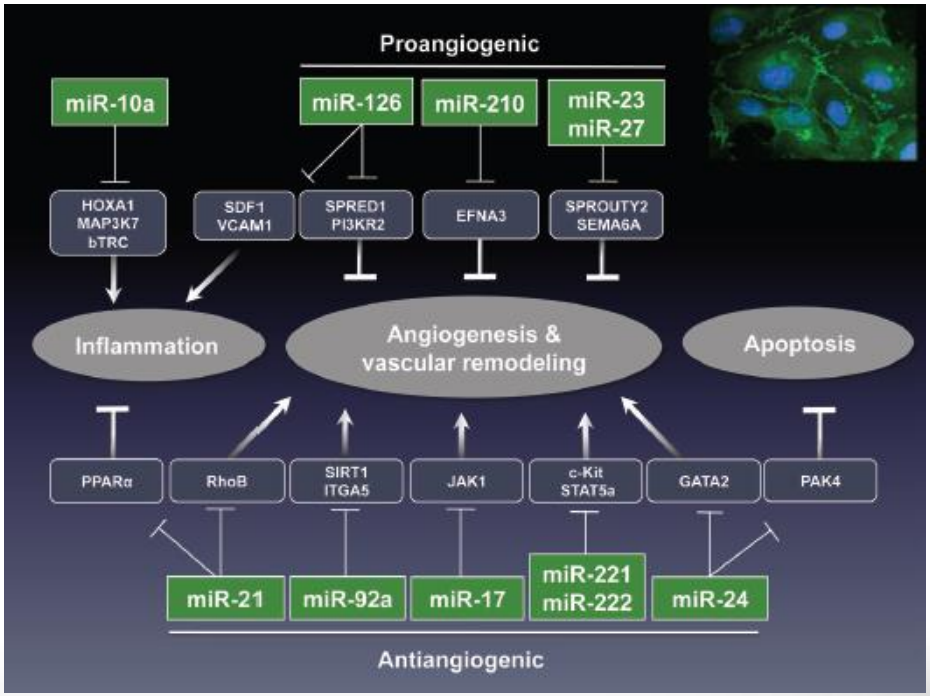
The widespread regulation of microRNA biogenesis, function and decay
Nature Reviews Genetics. 2010;11:597-610

Reciprocal negative feedback loop between miR-22 and c-Myc



Contents

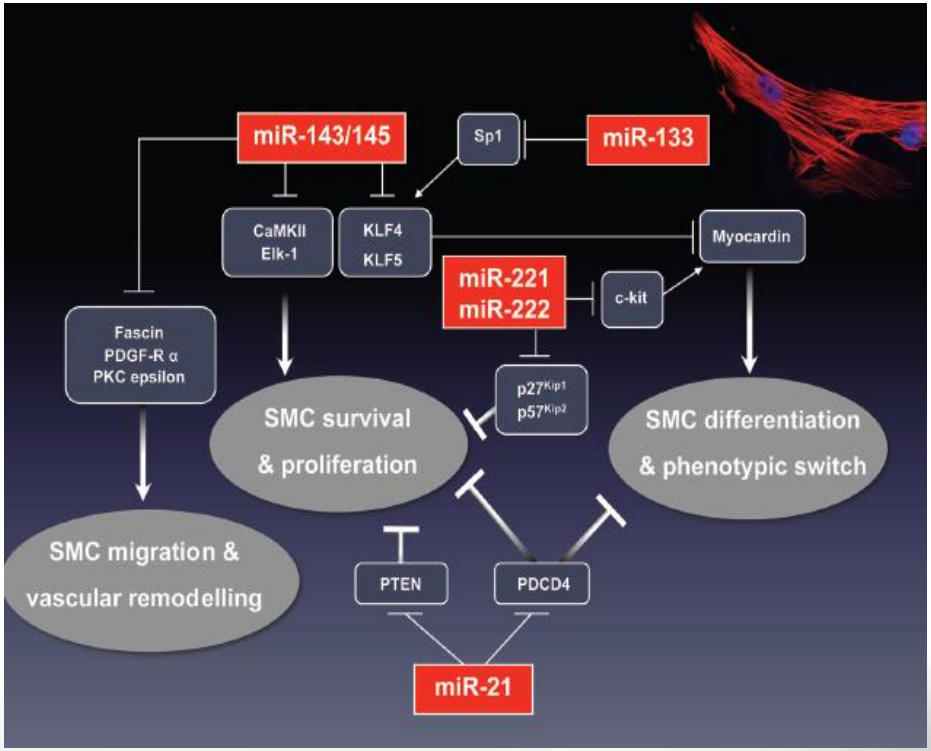
miRs involved in endothelial cell regulation



- EC-Specific Dicer Knockout
  - indistinguishable from littermate controls
  - Decreased Tie-1, -2, VEGF-R2
  - Increased eNOS
- MiR-17-92 Cluster
  - MiR-92a, an endogenous repressor of the angiogenic program
- MiR-126
  - Master regulator of EC function
  - Targeted deletion causing leaky vessels and hemorrhages
- KLF2 induces miR-126 but represses miR-92a
- Hypoxia induces miR-210, leading to angiogenesis

MicroRNAs in Vascular and Metabolic Disease. Review article. *Circ Res*. 2012;110:508-522

miRs involved in VSMC regulation

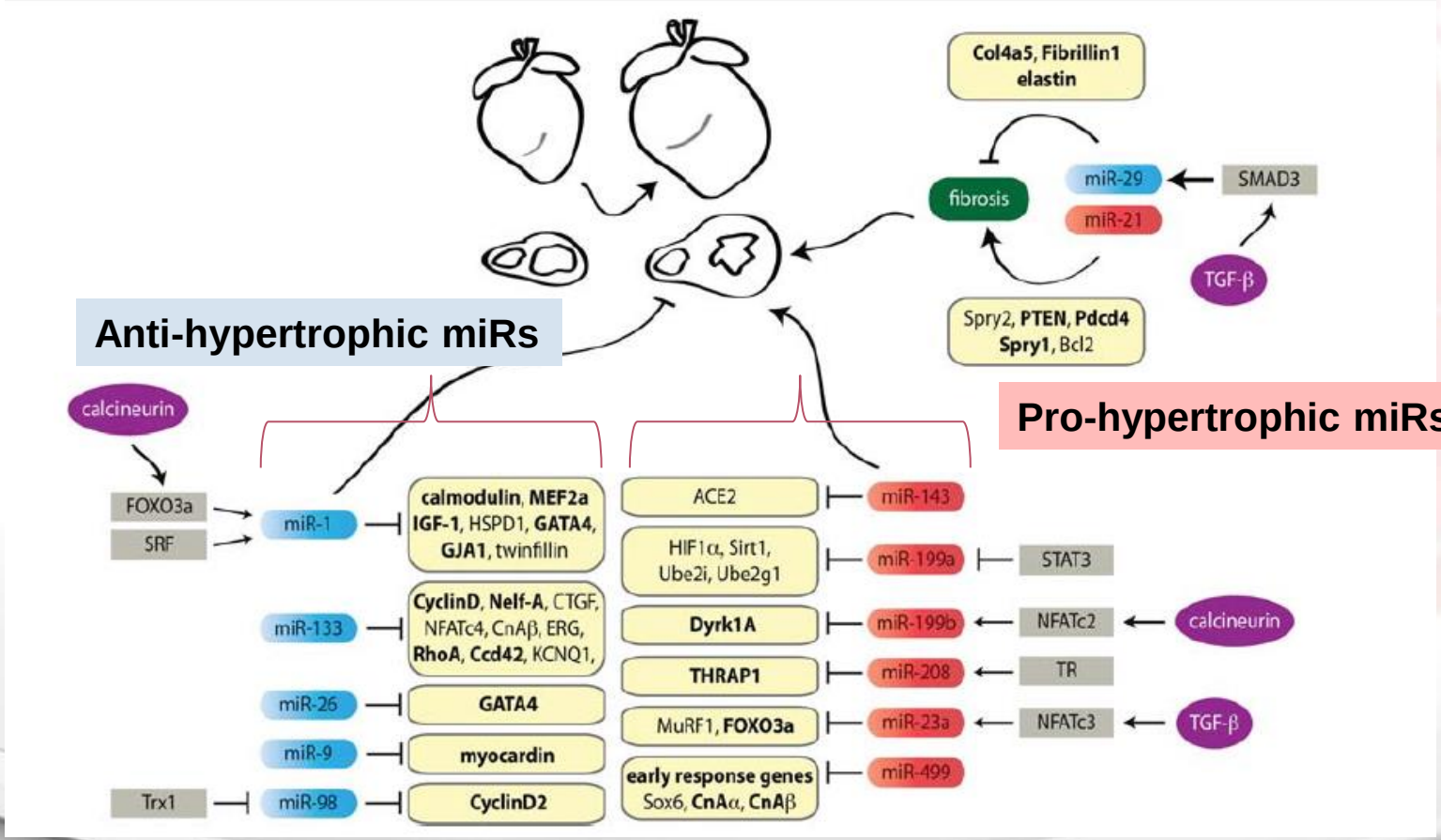


- SMC-Specific Dicer Knockout
  - – Embryonic lethal d/t extensive hemorrhages
  - – Dysorganized elastic lamellae
  - – Loss of actin, contractile dysfunction
- MiR-143/145 Cluster
  - – Downregulated in synthetic SMC
  - – Promoting a contractile phenotype
  - – Overexpression leading to reduced neointima formation
- MiR-21, MiR-133, MiR-221, and MiR-222
  - – Upregulated in neointimal lesions
  - – Promoting SMC proliferation while inhibiting apoptosis

MicroRNAs in Vascular and Metabolic Disease. Review article. *Circ Res*. 2012;110:508-522

miRs involved in cardiac hypertrophy

- 4 microRNAs highly expressed in the heart: “Myo-MiR”
  - **miR-1, miR-133, miR-208, miR-499**



MicroRNAs in control of cardiac hypertrophy.
Review article. Cardiovascular Research 2012;93:563–572

Anti-hypertrophic miR-1

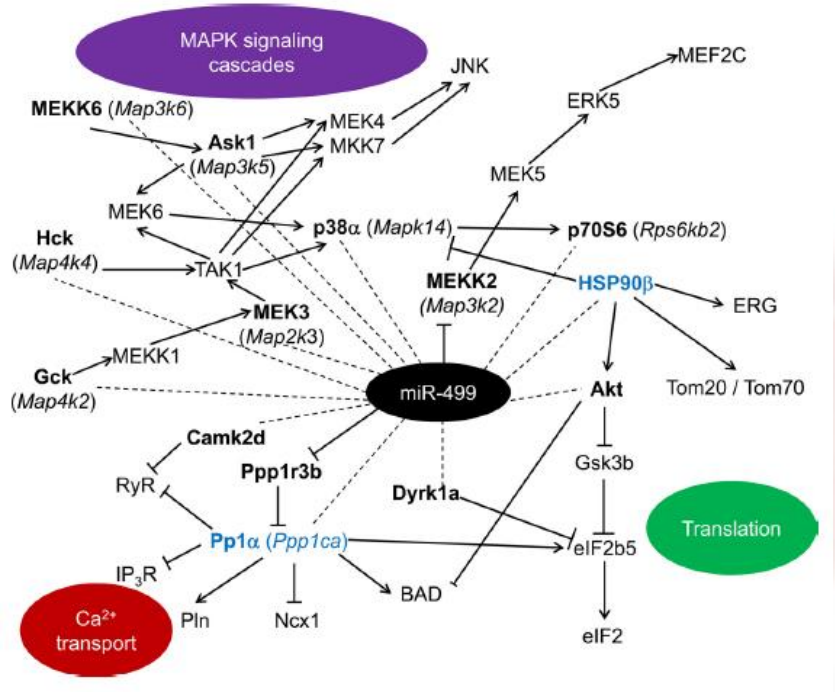
- Among the most abundantly expressed miRNAs in the human heart
- miR-1 overexpression causing developmental arrest due to dilated ventricles and heart failure at E9.0
- Validated targets of miR-1
  - Calcium signalling mediators, calmodulin
  - cytoskeletal regulatory protein twinfilin 1 (Twf1)
  - insulin-like growth factor (IGF-1)

Pro-hypertrophic miRs: miR-499, -208

- Pro-hypertrophic miRs located within MHC genes
  - miR-208a: *Myh6* encoding fast-twitch a-MHC
  - miR-208b: *Myh7* encoding slow-twitch b-isoform
  - miR-499: *Myh7b* encoding another fast-twitch isoform
- May play a crucial role in regulation of myosin gene expression and the **cardiac stress response**
- Deletion of miR-208a resulting in viable animals with normal cardiac size and function at baseline, but starting mild decline in cardiac function up to 5 months of age
- In response to cardiac stress, miR knockout hearts developed a greater cardiac dysfunction, without evident signs of cell hypertrophic growth or fibrosis.

miR-499, evolutionarily conserved muscle-specific miRNA

- Increased in human and murine cardiac hypertrophy and cardiomyopathy
- Sufficient to cause murine heart failure, and accelerates maladaptation to pressure overloading

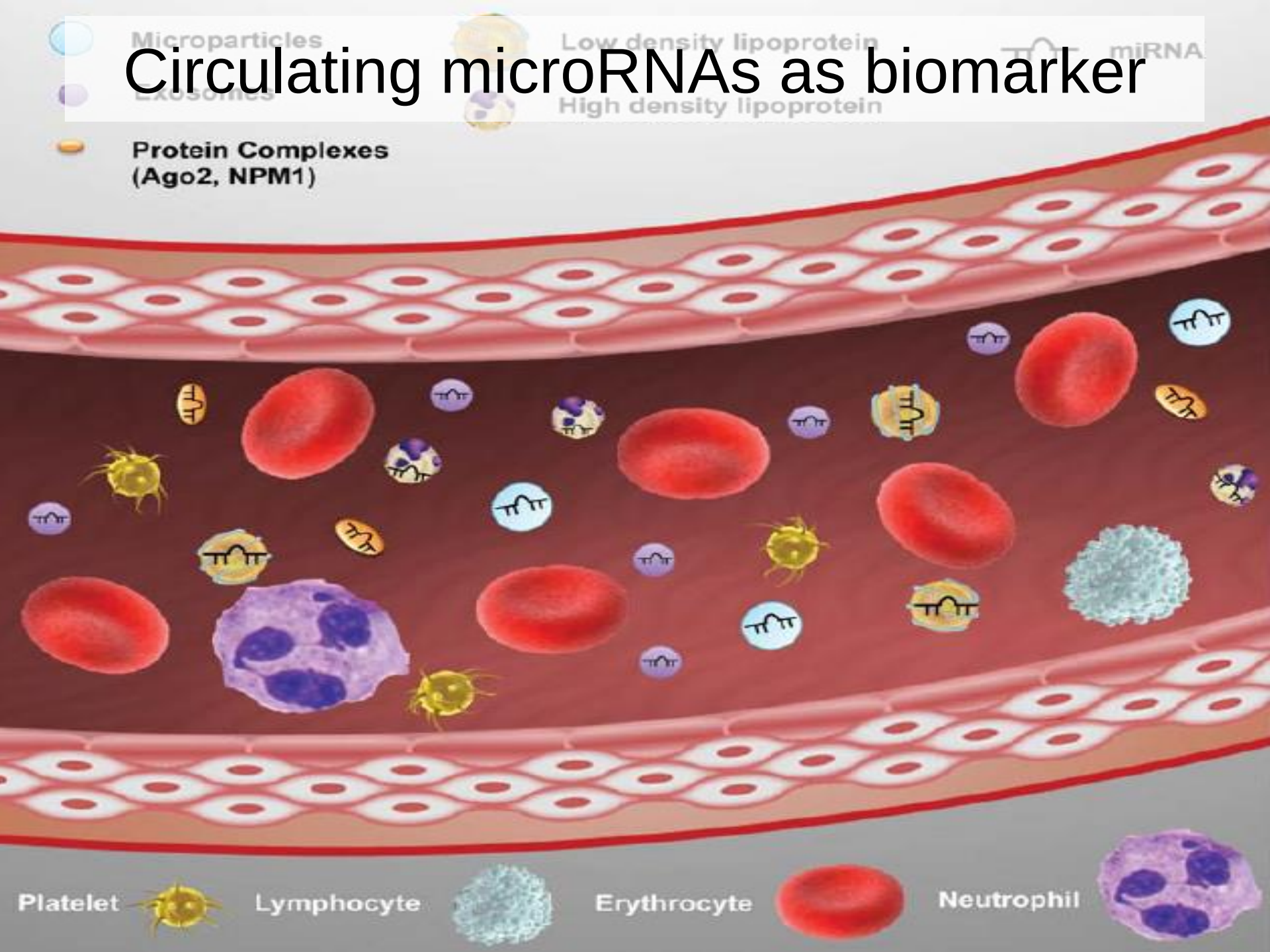


Direct and indirect involvement of MicroRNA-499 in clinical and experimental cardiomyopathy. Circ Res. 2012;111:521-531

miR-21 regulating pathological hypertrophy & fibrosis

- Contributing myocardial remodelling through regulation of the ERK-MAP kinase-signalling pathway, a crucial signalling pathway in fibroblast survival and activation
- Strongly induced in the failing myocardium and mostly predominant in **fibroblasts**
- Silencing preventing cardiac dysfunction in a mouse model of cardiac pressure overload
- Overexpression inducing interstitial fibrosis and cardiac hypertrophy

Circulating microRNAs as biomarker

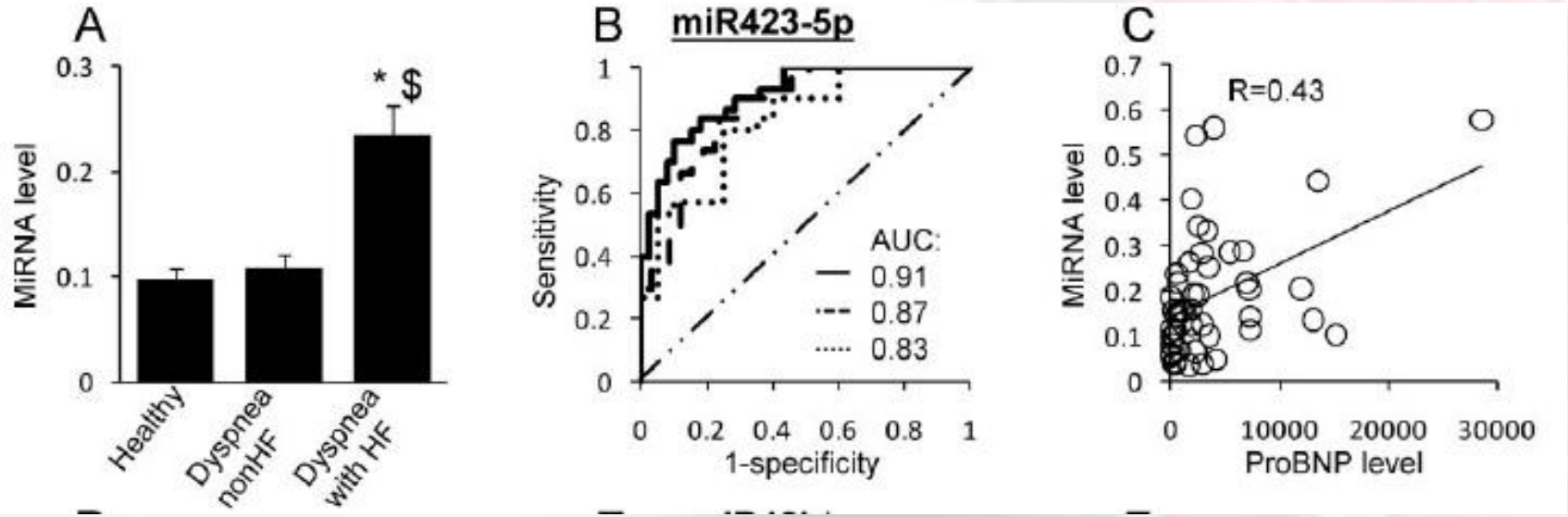


Brief UltraRapid Communication

MiR423-5p As a Circulating Biomarker for Heart Failure

Anke J. Tijsen,* Esther E. Creemers,* Perry D. Moerland, Leon J. de Windt, Allard C. van der Wal, Wouter E. Kok, Yigal M. Pinto

(*Circ Res*. 2010;106:1035-1039.)



In the HF group, more than one-third had an ejection fraction of 45% (11 of 30 subjects), suggesting preserved systolic function (HFpEF). Preliminary evidence suggests that miR423-5p levels were elevated similarly in both patient groups.

Circulating microRNAs evaluating etiologic mechanism / disease progress of heart failure

- From AMI Pts, the level of miR-208b, -499, -1 level significantly elevated. When Pts with recent cardiac ischemia or infarction excluded, no increases in those miRs found in HF patients.
- EC-specific miR-126 negatively correlated with age, BNP, and NYHA class in HF. miR-126 also decreased in atherosclerotic CAD, & in Pts with type 2 DM, reflecting the condition of vascular endothelial cells in HF Pts.
- 72% of miRs differentially regulated in LVAD treated patients normalized after treatment

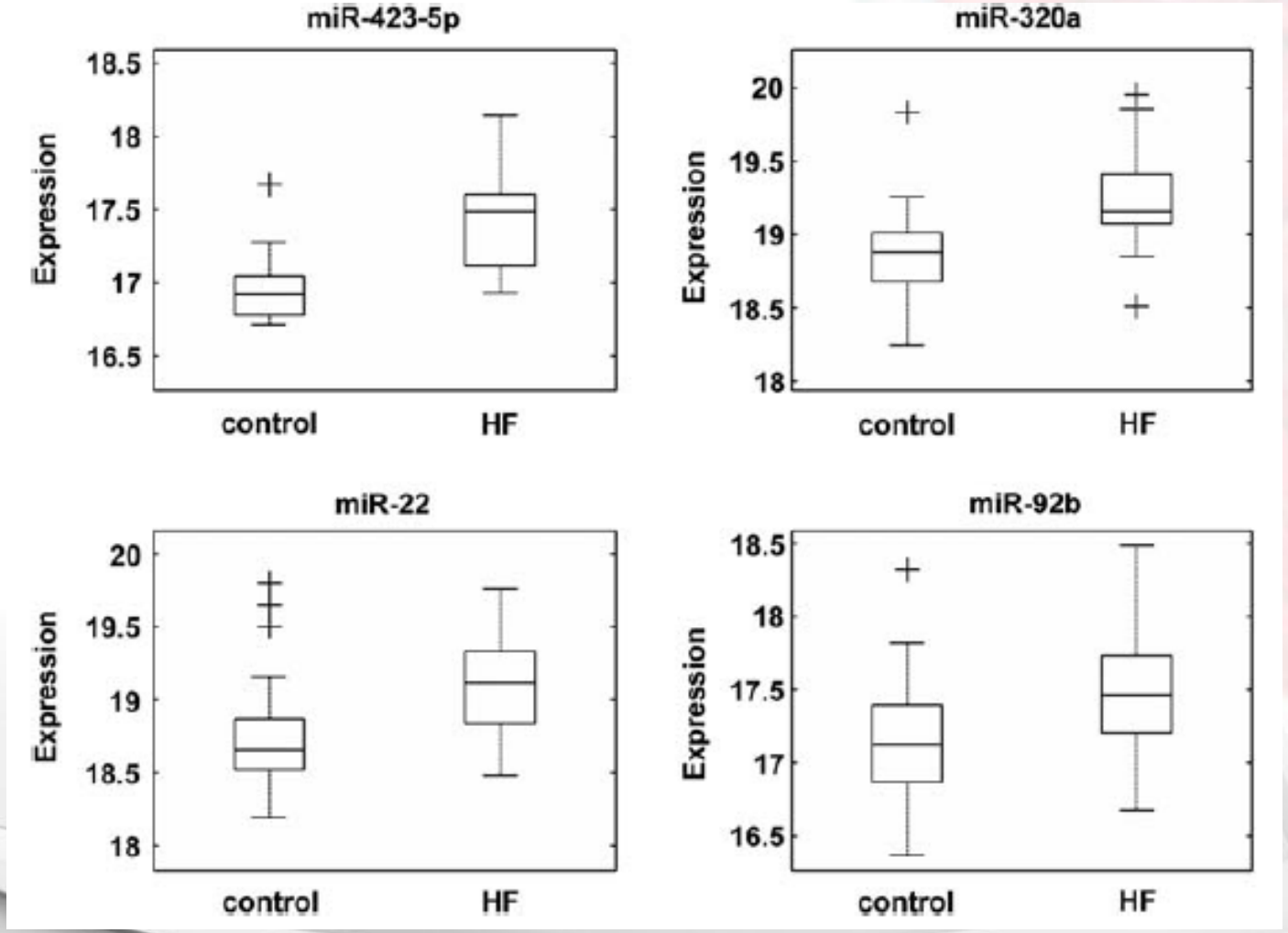
Circ Res. 2012;110:483-495

MicroRNA with increased levels in sera of heart failure patients

miRNA	P-value	Fold change	AUC[a]
miR-423-5p	1.80E-08	1.5	0.88
miR-320a	1.50E-05	1.2	0.86
miR-22	1.30E-04	1.4	0.80
miR-92b	4.50E-04	1.3	0.76
miR-17	7.50E-04	1.3	0.76
miR-532-3p	8.20E-04	1.4	0.73
miR-92a	1.90E-03	1.4	0.74
miR-30a	2.90E-03	1.4	0.73
miR-21	4.20E-03	1.3	0.72
miR-101	7.20E-03	1.4	0.71

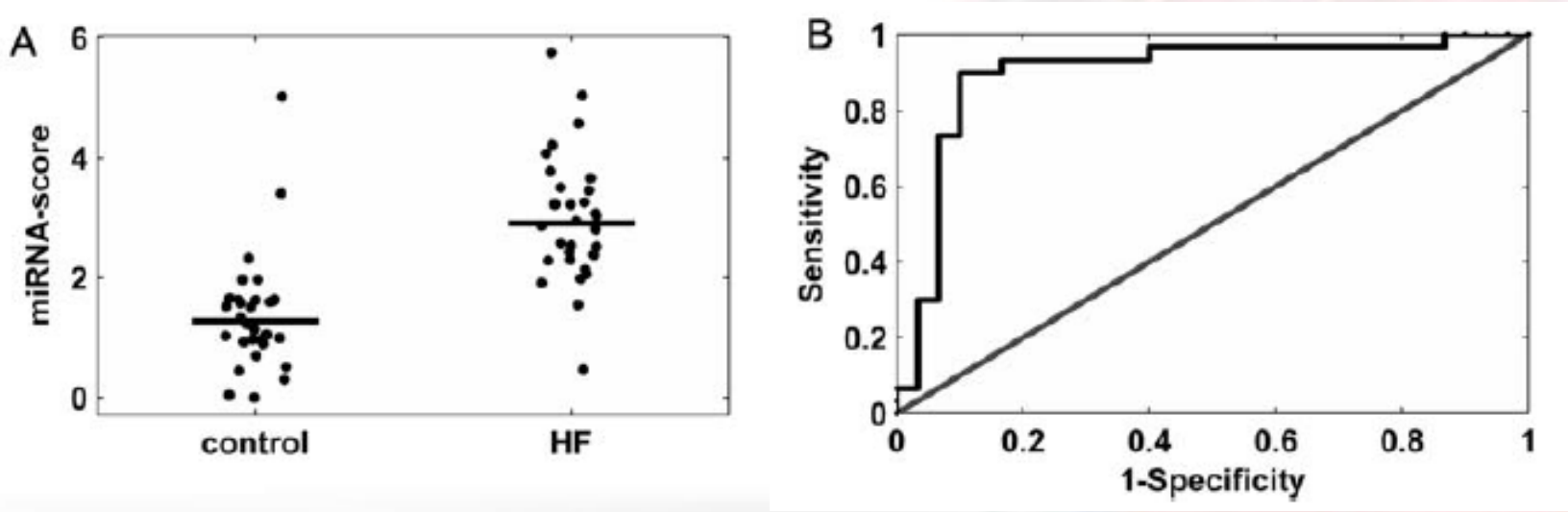
European Journal of Heart Failure (2012) 14, 147–154

MicroRNA with increased levels in sera of heart failure patients



European Journal of Heart Failure (2012) 14, 147–154

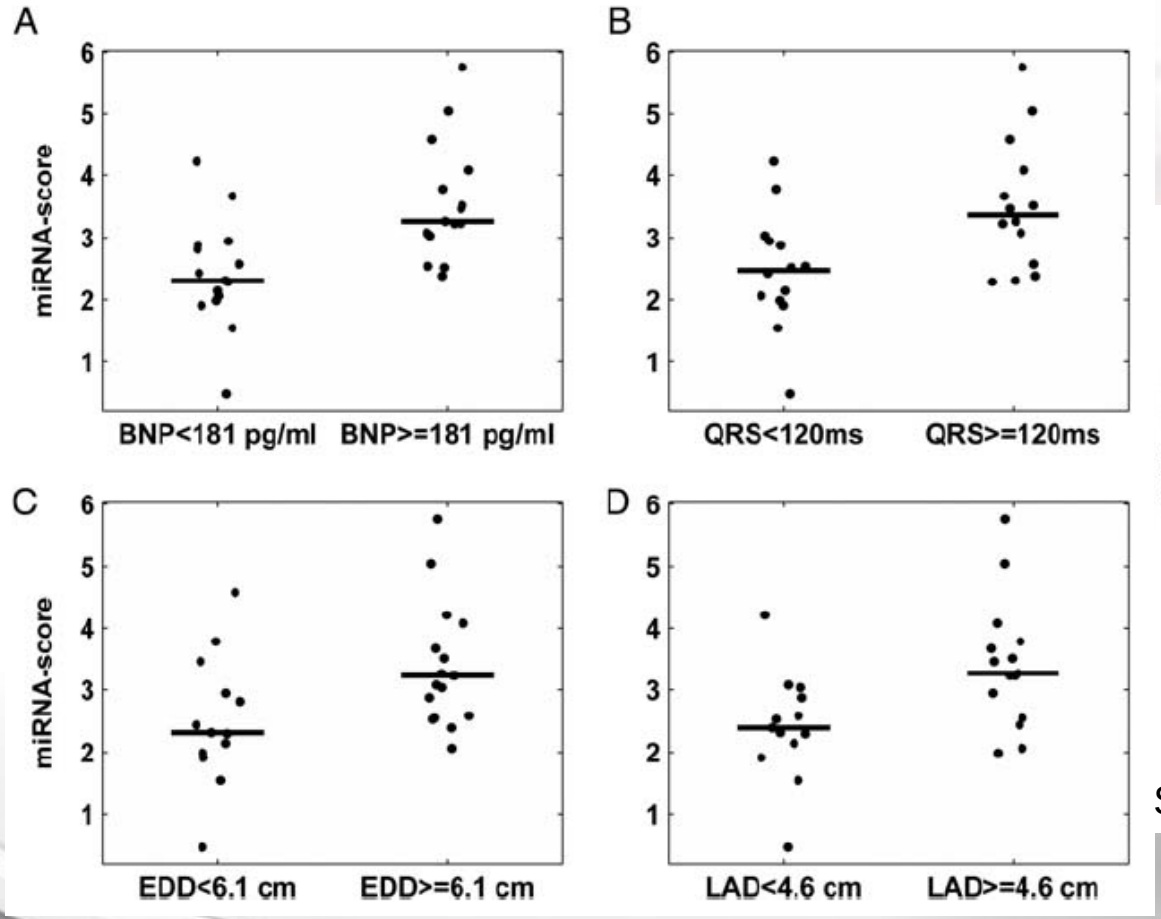
Discrimination between the heart failure and control groups using the microRNA score

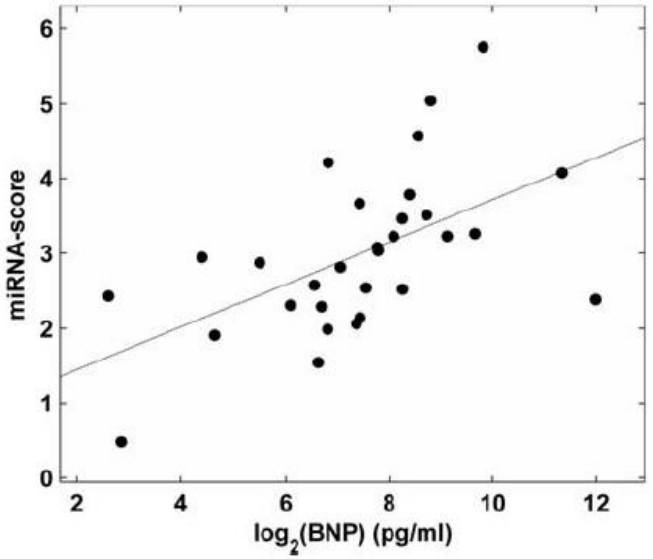


miRNA-score: cumulative level of the four miRNAs with the most significantly increased levels in the HF group when compared with the control

European Journal of Heart Failure (2012) 14, 147–154

Significant associations between the microRNA (miRNA) score and clinical parameters





Spearman correlation: 0.63 (P=3e-4)

Circulating miRNAs have many requisite features of good biomarkers

- Stable in various bodily fluids
- Sequences of most miRNAs conserved among different species
- Expression of some miRNAs specific to tissues or biological stages
  - using specific miRNA levels in blood to detect drug-induced liver injury
- Compared with different post-translational modifications of protein-based biomarkers, miRNA are relatively homogenous.
- Polymerase chain reaction (PCR) measurement is more sensitive than ELISA
- Readily available antagomir for miR regulation

A. Etheridge et al. Mutation Research 2011;717:85– 90

Issues associated with miRNA measurement.

- **Measurement**
  - Low correlation between different measurement platforms
  - Short and conserved sequences in paralogs
  - Difficult to distinguish between precursor and mature forms
- **Sample**
  - Concentration measurement for miRNA in sample is difficult
- **Data processing**
  - Normalization among different samples, especially for extracellular miRNA
- **Predictive**
  - whether circulating miRNAs track with disease progression?
  - whether changes in miRNAs are indicative of therapeutic efficacy?

A. Etheridge et al. Mutation Research 2011;717:85– 90

Contents

Angiotensin II and microRNA

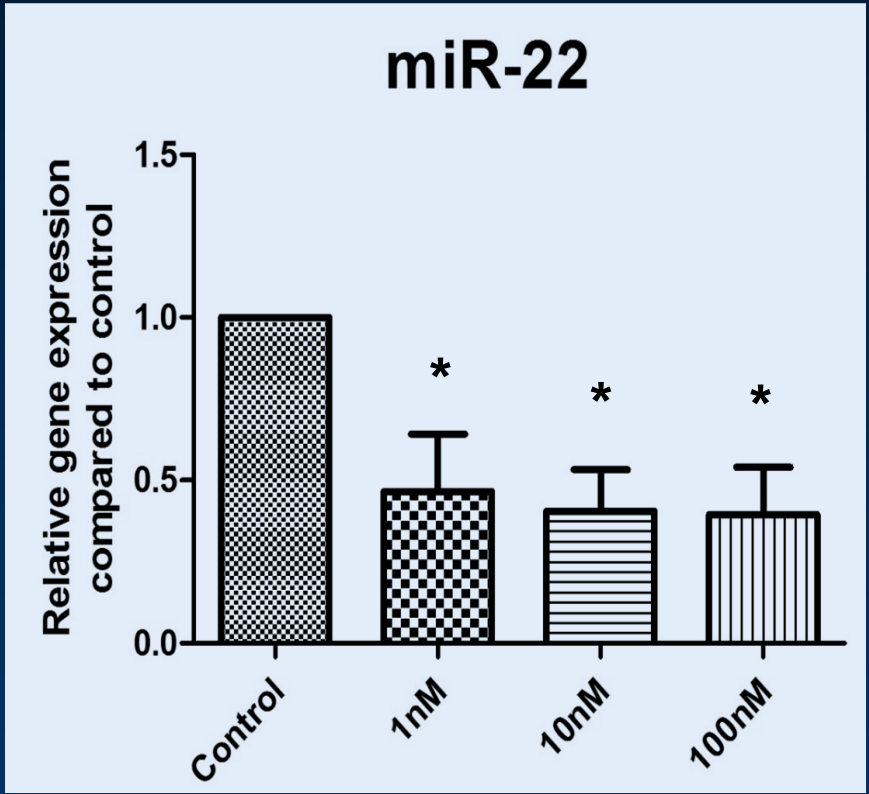
- Predicted miRs that target the 3'-UTR of CYR61: TargetScan & miRANDA

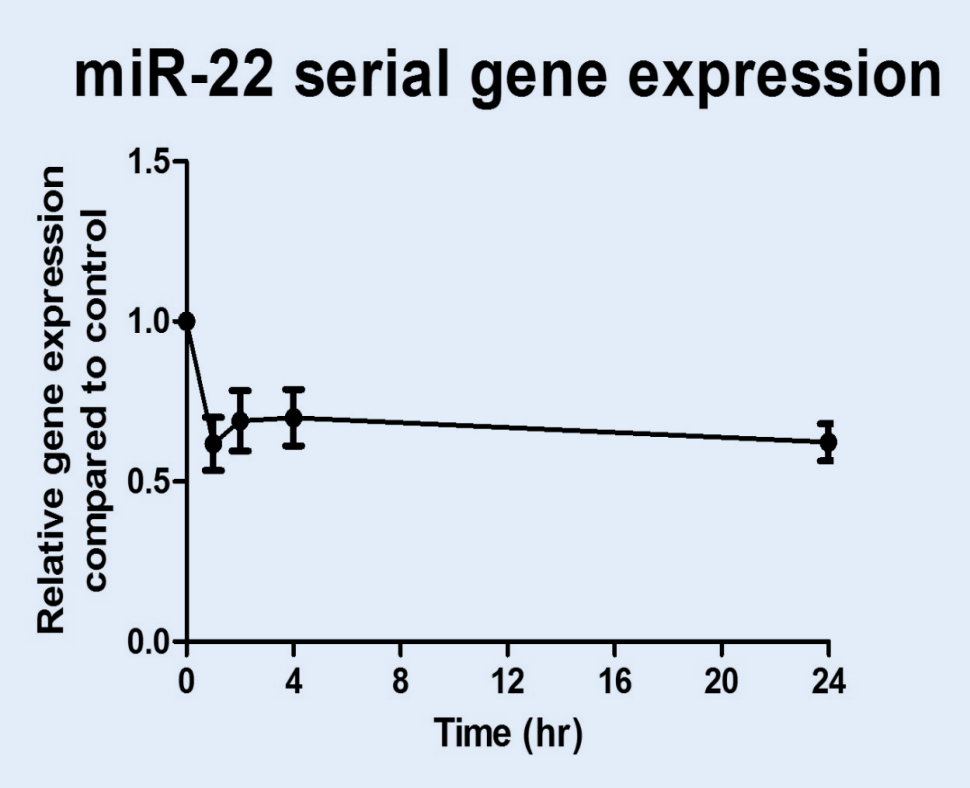
- miR microarray of angiotensin II-treated VSMCs

Candidate miRs that were downregulated by Angiotensin II

miR-145
miR-22
miR-181a
miR-340
miR-221
miR-181d

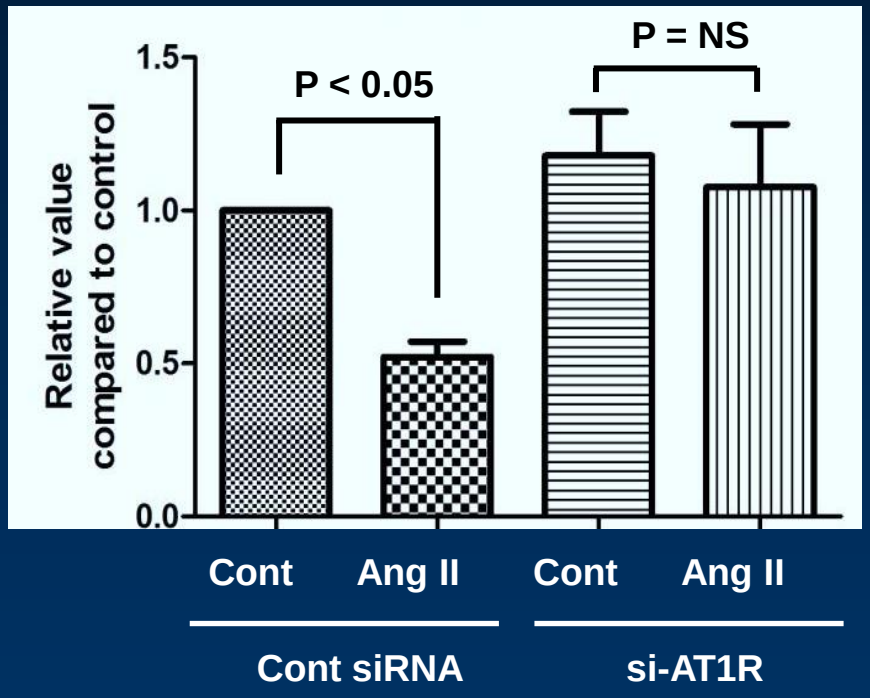
Angiotensin II induces sustained downregulation of miR-22

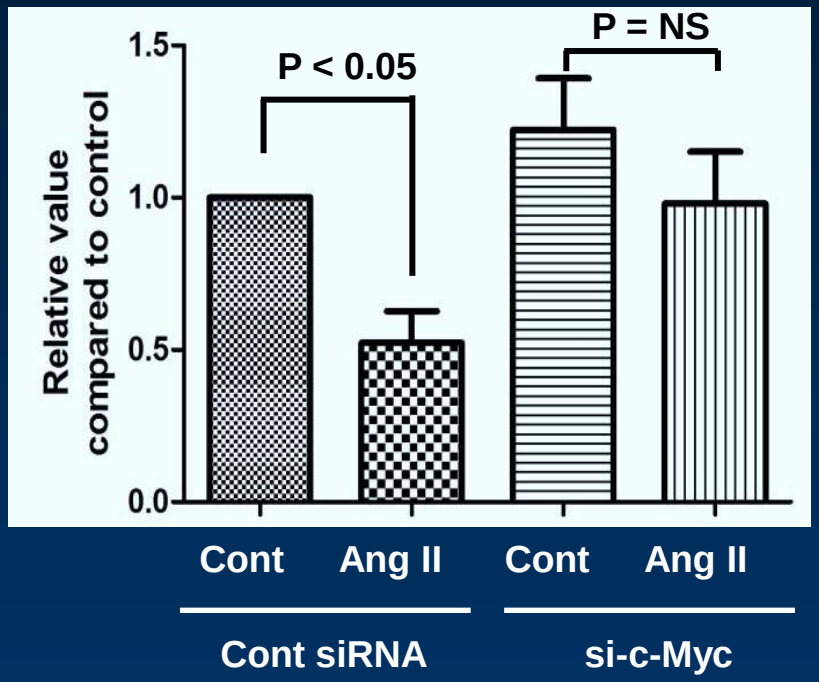




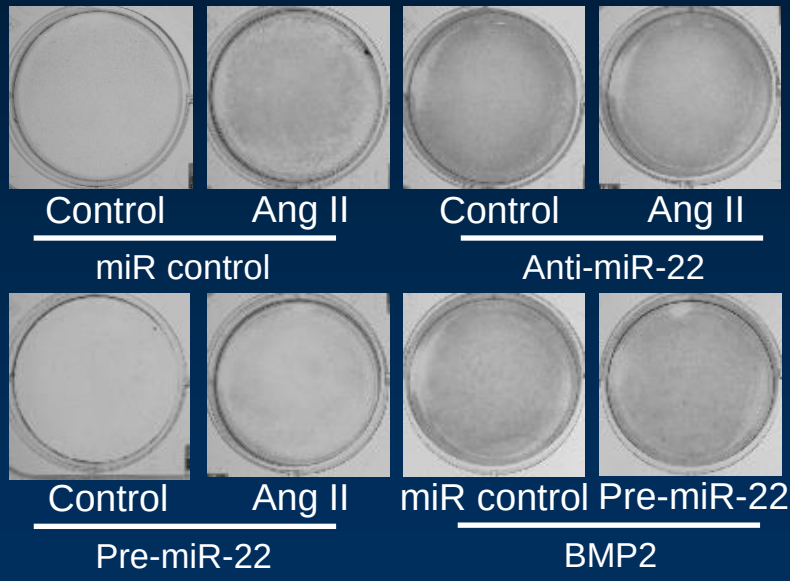
*p < 0.05 compared to control
4 h after angiotensin II treatment

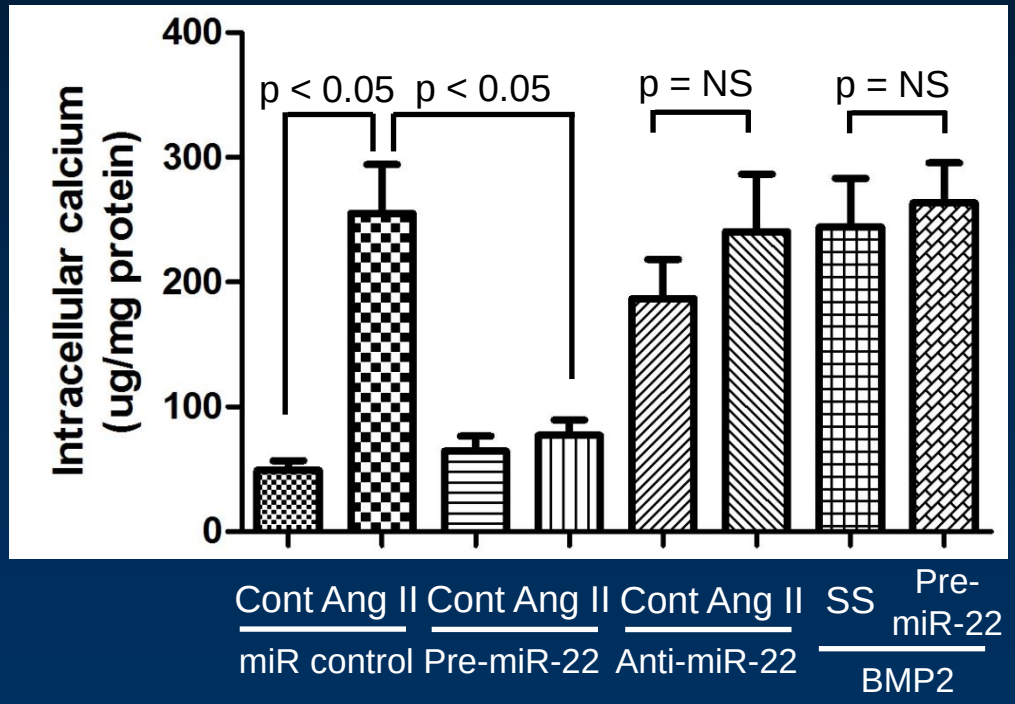
Angiotensin II downregulates miR-22 expression via AT1R-cMyc





Angiotensin II downregulates miR-22 to promote calcification

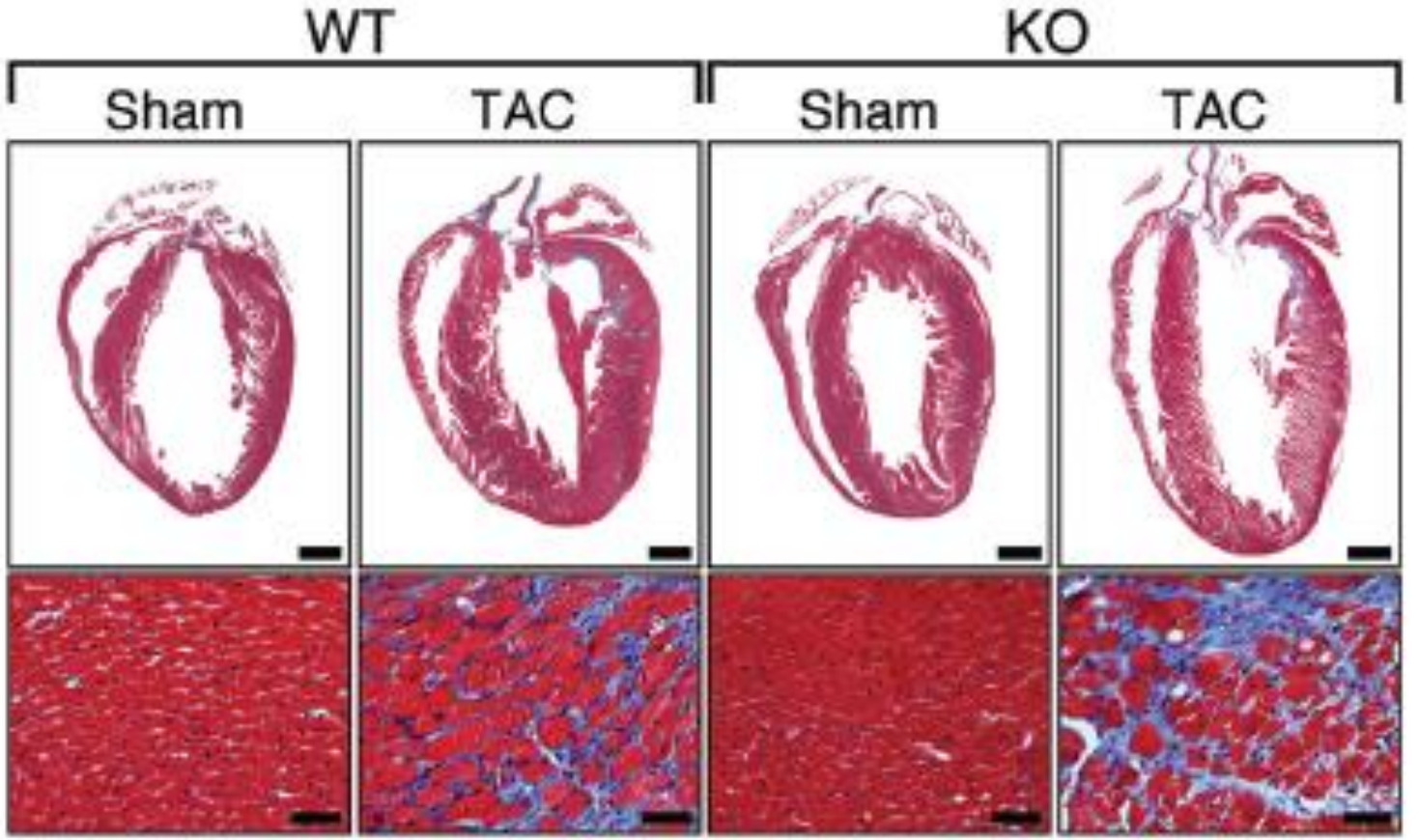




Targeted Deletion of MicroRNA-22 Promotes Stress-Induced Cardiac Dilation and Contractile Dysfunction

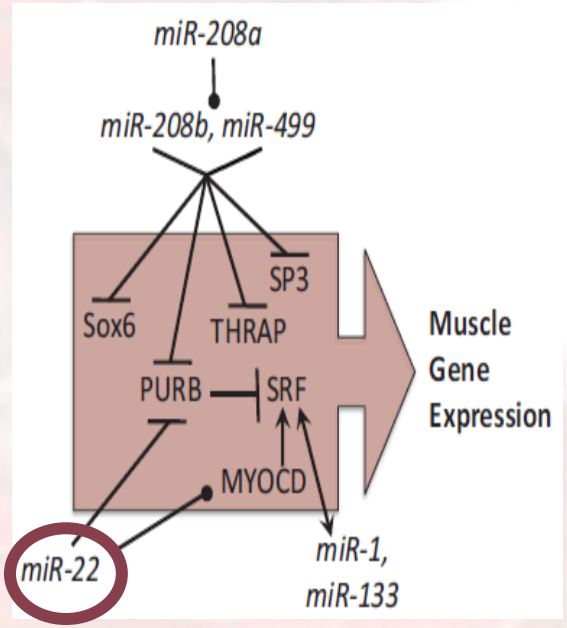
Conclusion—These data indicate that *miR-22* functions as an integrator of Ca^{2+} homeostasis and myofibrillar protein content during stress in the heart and shed light on the mechanisms that enhance propensity toward heart failure. (***Circulation*. 2012; 125:2751-2761.**)

- miR-22 functions as an integrator of calcium homeostasis in heart?



miR-22 as a critical regulator of cardiomyocyte hypertrophy and cardiac remodeling

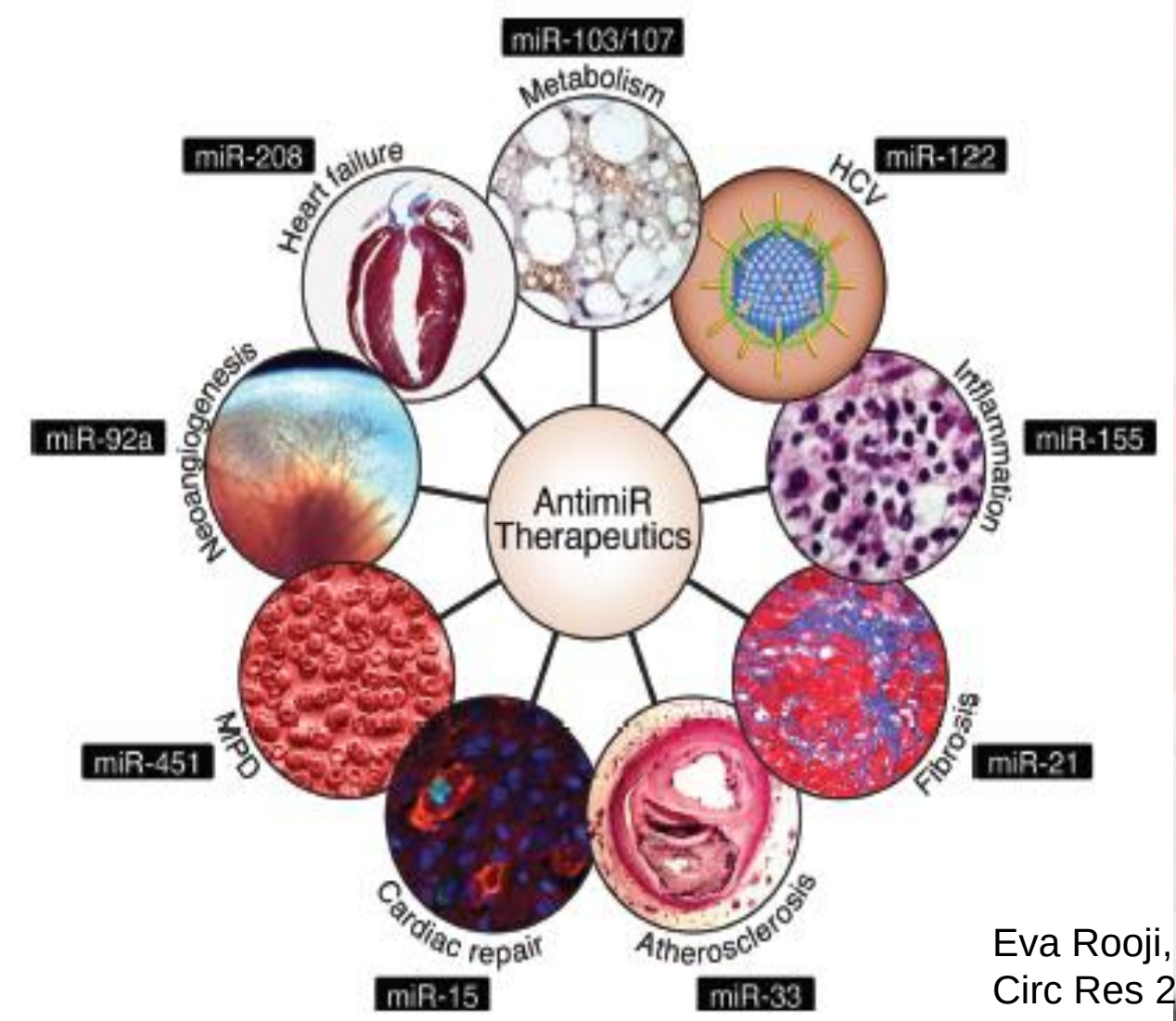
- Cardiac and skeletal muscle enriched microRNA upregulated during myocyte differentiation and (physiologic) hypertrophy
- Overexpression sufficient to induce cardiomyocyte hypertrophy
- Global and cardiac-specific deletion showing essential role for hypertrophic cardiac growth in response to stress
- Deletion had little impact on normal hearts
- Deleted mice sensitized to development of D-CMP under stress conditions
- 50% of null mice died in utero (some with cardiac defects), suggesting housekeeping/developmental function



Circ Res. 2013

Contents

MicroRNAs are the hottest topics in cardiovascular research



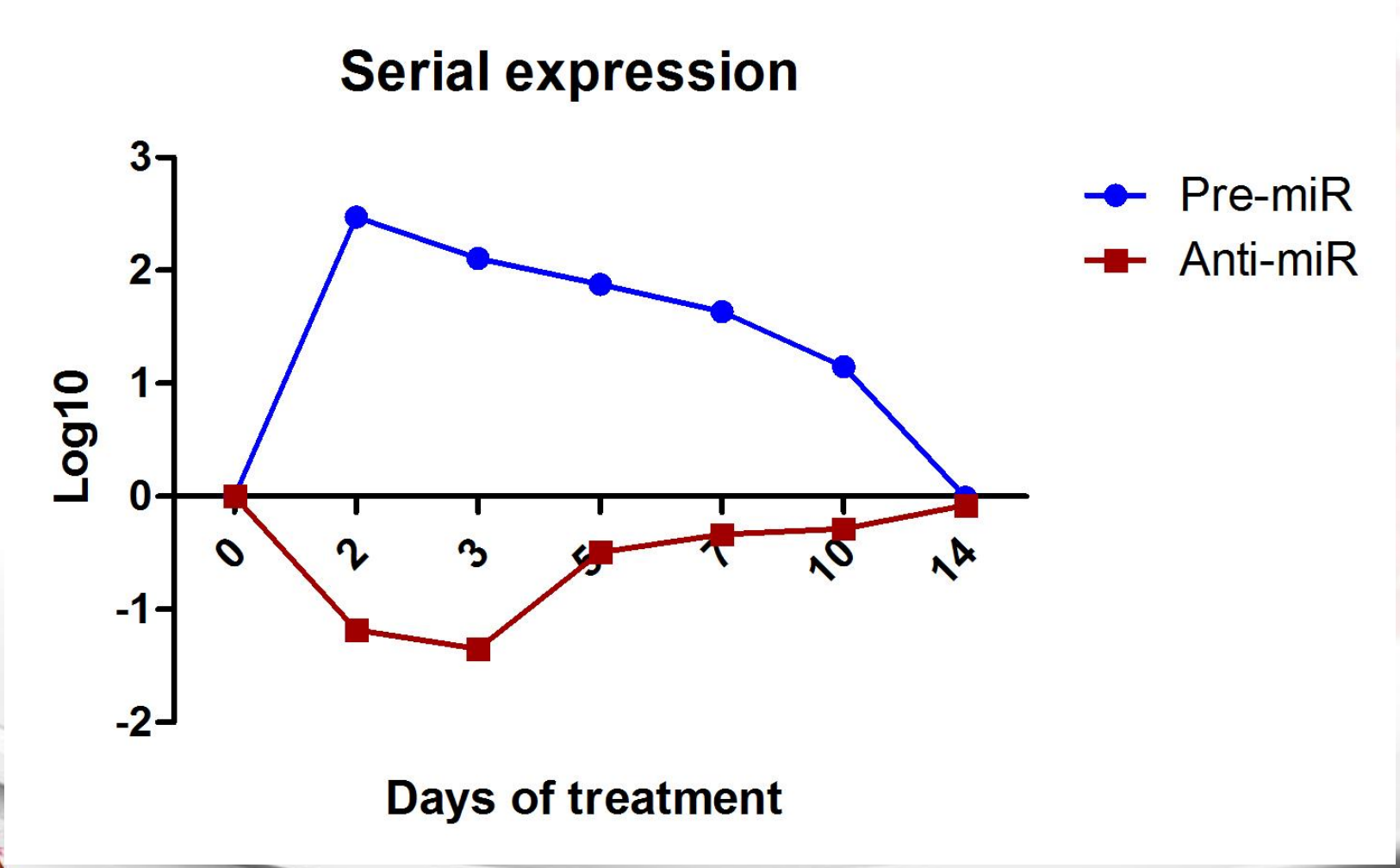
Eva Rooji, Arthur A Levin
Circ Res 2012

Developing microRNA therapeutics

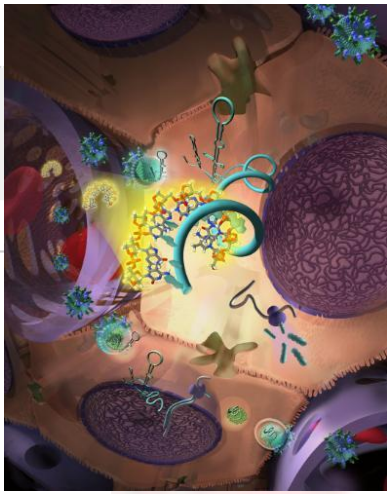
- **Anti-miR**
  - Modified **antisense oligonucleotides** harboring the full or partial complementary reverse sequence of a mature miRNA, reducing endogenous levels of an miRNA
- **PremiR**
  - artificial small nucleotide sequences, double-strand, similar to miRNA precursors
- **Benefit of microRNA pharmacodynamics**
  - anti-miR are **cleared from plasma within hours** by uptake into tissues. But, **inside cells, anti-miRs are so metabolically stable** that their clearance is slow, and half-lives in tissues are often reaching weeks
  - Because of their **high water solubility**, it is possible to dissolve anti-miRs in aqueous solutions at volumes that are amenable to administration by the subcutaneous or parenteral route.
- **Limitation to be solved**
  - Off-target effect: **exaggerated pharmacology** making toxicity resulting from binding and inactivating unintended target or acting **in inappropriate cells**

Eva Rooji, Arthur A Levin Circ Res 2012;110:496-507

Forced-expression experiment : Duration of forced-expressed miRNA



Santaris Pharma A/S Phase 2a Data of Miravirsen Shows Dose-Dependent, Prolonged Viral Reduction of 2-3 Logs HCV RNA After Four-Week Treatment in Hepatitis C Patients



- New Phase 2a clinical data to be presented in late-breaking oral presentation at AASLD -

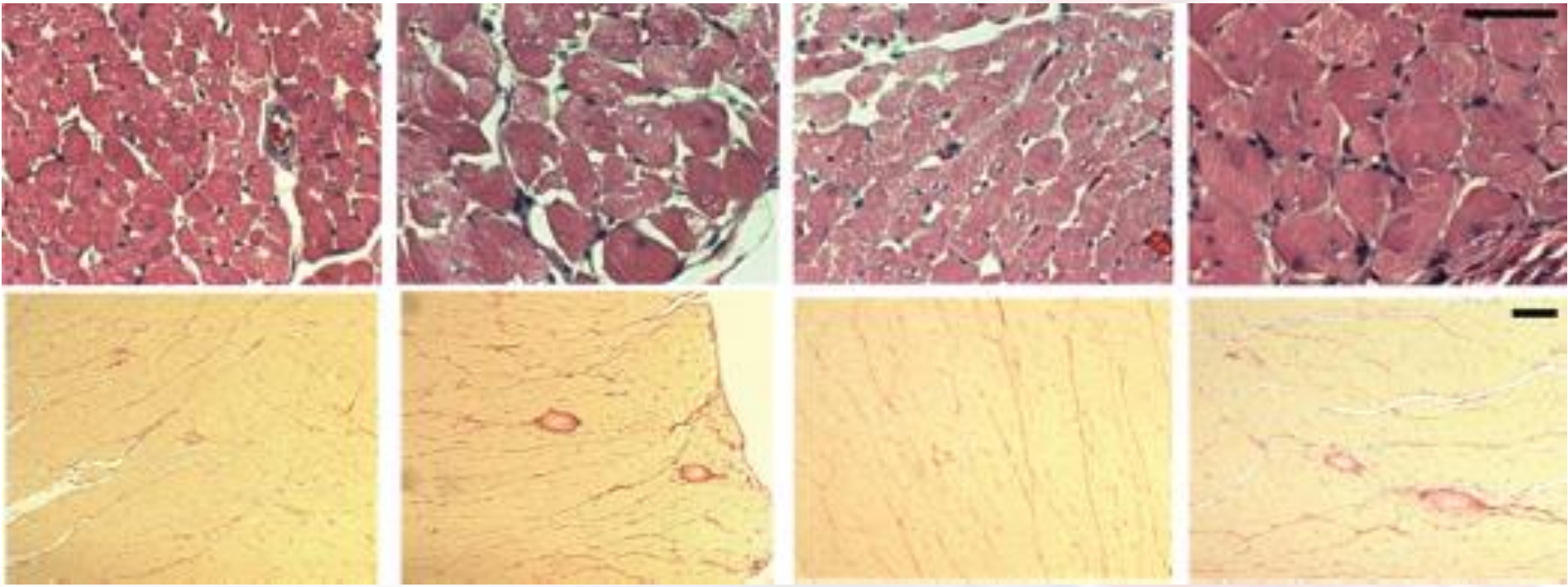
- Miravirsen given as a four-week monotherapy treatment provided robust, dose-dependent antiviral activity with a mean reduction of 2 to 3 logs from baseline in Hepatitis C Virus (HCV) RNA (log10 IU/mL) that was maintained for more than four weeks beyond the end of therapy

- Four out of nine patients treated at the highest dose with miravirsen became HCV RNA undetectable during the study, providing clinical evidence that miravirsen's unique mechanism-of-action offers high barrier to viral resistance and the potential for treatment cures with monotherapy

- Miravirsen, the first microRNA-targeted drug to enter clinical trials, works by inhibiting miR-122, a microRNA required for HCV accumulation, was well tolerated in patients with chronic HCV infection

- Miravirsen's long-lasting suppression of HCV RNA, high barrier to viral resistance, low propensity for drug interactions and favorable tolerability profile holds promise as pivotal new treatment option given as monotherapy or in combination with direct acting antiviral agents as an interferon-free treatment to eradicate chronic HCV infection in multiple genotypes

Therapeutic Inhibition of miR-208a improves cardiac function and survival during heart failure



Dahl hypertensive rat with low-salt diet

Dahl hypertensive rat with 8% high-salt diet

Dahl hypertensive rat with 8% high-salt diet + SC anti-miR208a

Dahl hypertensive rat with 8% high-salt diet + SC control

Subcutaneous delivery of anti-miRs

Circulation. 2011;124:1537-1547

Use of myo-microRNAs in the fight against heart failure

microRNA	Usefulness	Effect
miR-21	Therapy via inhibition by antagomir	Significant regression of cardiac hypertrophy and fibrosis and attenuation of impairment of cardiac function.
miR-208a	Biomarker of acute myocardial injury	Its circulating levels increases after myocardial injury. Detected earlier than cardiac troponin.
	Therapy via inhibition by antagomir	Therapeutic inhibition of miR-208a avoided the pathological myosin changes and cardiac remodeling, improving cardiac function and increasing their survival
miR-423-5p	Biomarker of HF	Its increased levels during HF make them a strong predictor of HF.
miR-499	Biomarker of acute myocardial injury	Its circulating levels increases after myocardial injury.

Mol Biol Rep. 2013;40:2663–2670

Summary and conclusion

- microRNAs are suggested to regulate > 60% of all human genes; Single target gene regulated by multiple microRNAs, single microRNA regulates multiple genes.
- Recently, the crucial role of myo-miRNAs has been widely recognized including pro-hypertrophy, anti-hypertrophy, pathologic remodeling and fibrosis.
- Circulating miRNAs are remarkably stable, raising the possibility as novel diagnostic markers.
- MicroRNA therapeutics are the hottest topic in cardiovascular research especially in terms of microRNA silencing using anti-miR.
- Systems biology approach is being pursued in order to integrate microRNA networks regulating multiple targets in disease pathway.