THE UNIVERSITY OF CHICAGO MEDICINE

Heart and Vascular Center

Micro-RNA Therapeutics in Cardiovascular Disease: Where does the Science Lie?

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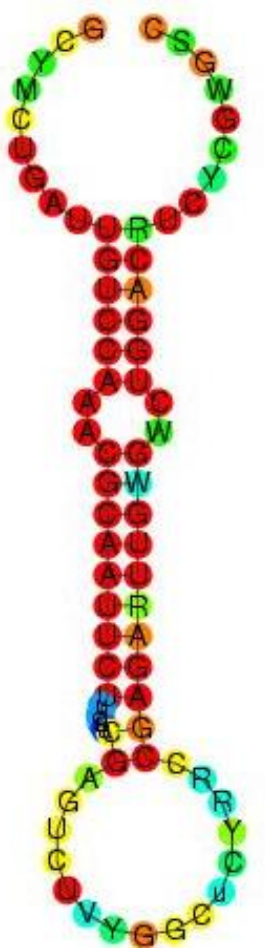
Disclosures

- No relevant financial disclosures
- No off-label therapeutic discussion

Objectives

- Discuss mechanisms of micro-RNA (miRNA) inhibition of protein translation
- Introduce miRNAs as a new class of biomarkers in cardiovascular disease
- Review current and potential applications for miRNA technology in cardiovascular disease
- Highlight potential and known adverse effects of RNA technology in the treatment of cardiovascular disease

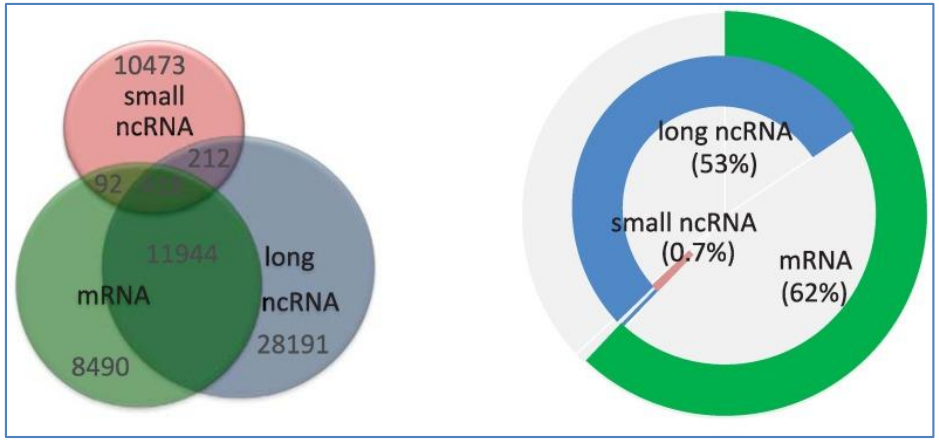


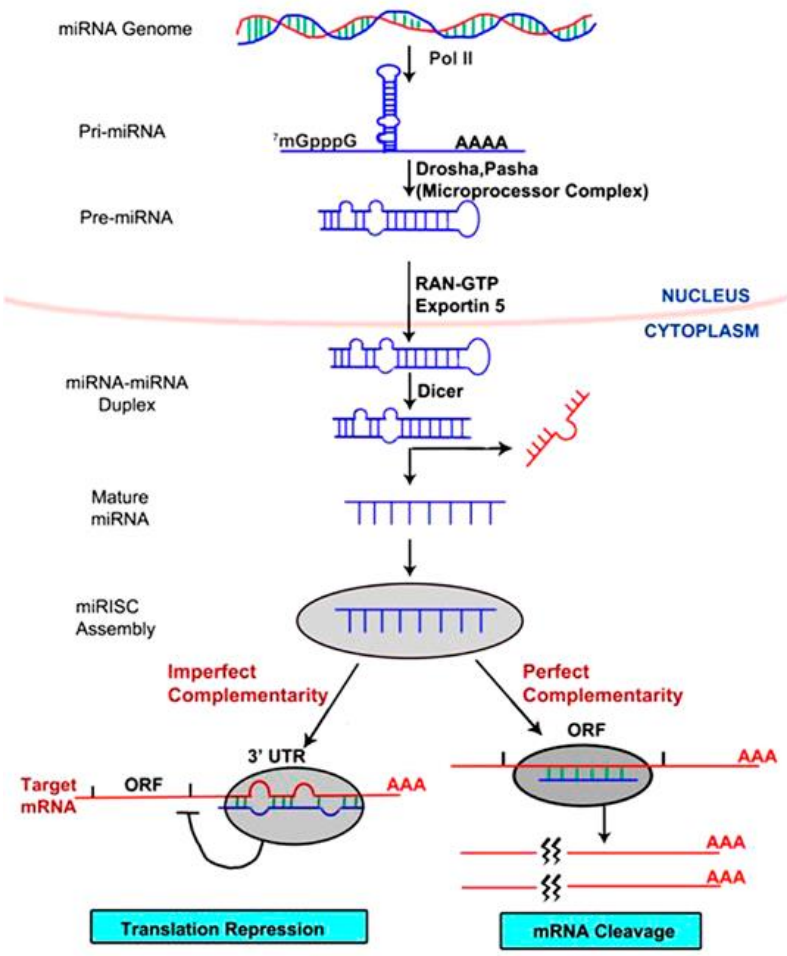
miRNA – Mechanisms for Repression

Evolutionarily conserved ncRNA approximately 20–22 nucleotides in length

Inhibit expression of native genes through direct binding to multiple mRNAs

May also induce RNA degradation when sequences match



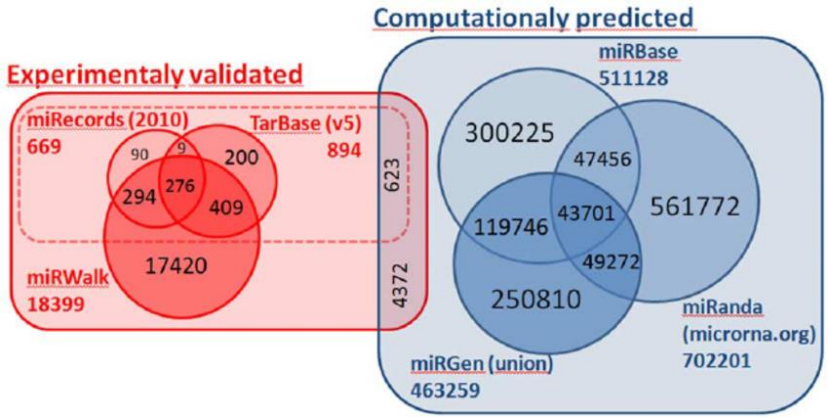


Mack ,et al. Nature Biotechnology **25**, 631 - 638 (2007)
Pertea, et al. Genes (Basel). 2012 Sep; 3(3): 344–360.

miRNA Diversity and Plurality

To date, over 1880 unique human miRNAs have been identified

May target and inhibit <u>hundreds-thousands</u> of genes



Kozomara, et al. Nucl. Acids Res., 39 (2011), pp. D152–D157

miRNA – A Novel Class of Biomarkers

miRNAs are known to be released from dying cells

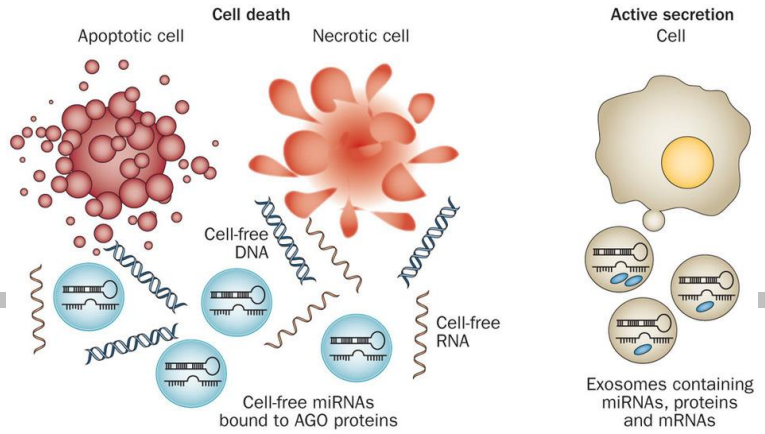
miRNAs are also actively secreted from multiple cell types

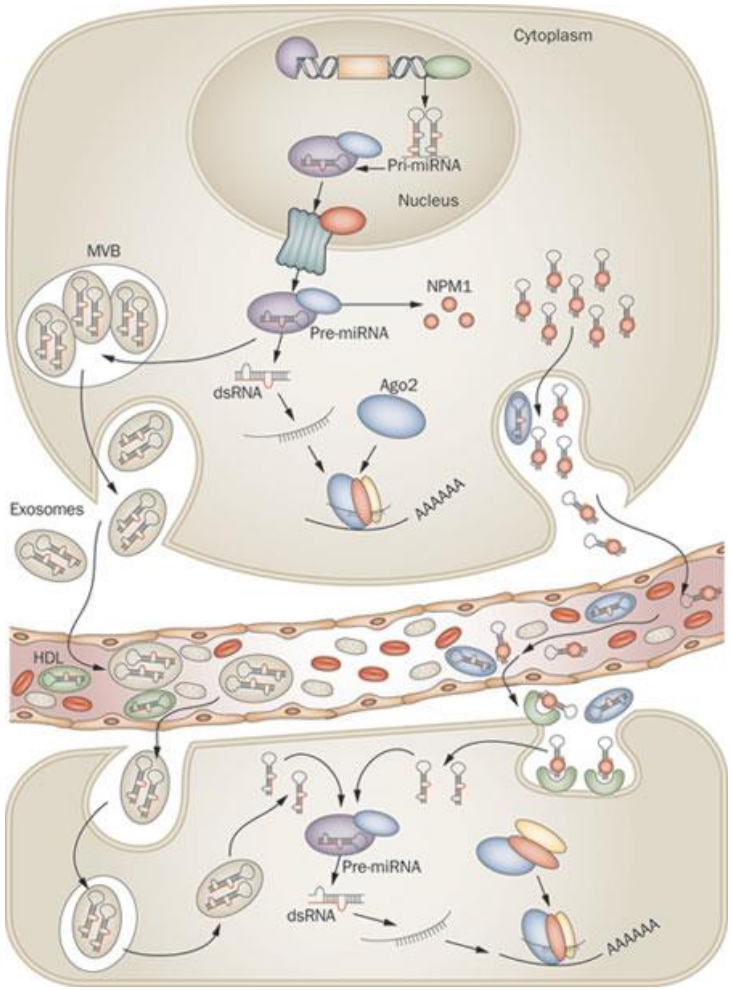
Can miRNA levels in the blood detect and/or predict cardiovascular disease?

mRNAs are protected from RNAse and other forms of degradation

- packaging in extracellular vesicles
- association with RNA-binding protein complexes
- in lipoproteins including HDL and LDL

mechanisms that govern the release of miRNA into the extracellular space are incompletely understood





Nature Reviews Clinical Oncology **8**, 467-477 (2011)
Nature Reviews Clinical Oncology **11**, 145–156 (2014)

Cardiovascular Disease and miRNA

miRNA expression patterns differ between healthy subjects and those with CVD and other cardiovascular diseases

mRNAs are protected from RNAse and other forms of degradation

- packaging in extracellular vesicles

- association with RNA-binding protein complexes

- in lipoproteins including HDL and LDL

mechanisms that govern the release of miRNA into the extracellular space are incompletely understood

Groups	miRNA	Source	Sample	Expression
Tobacco smoke exposure	miR-126-3p, 5p	Human	Plasma	Up-regulation
	miR-126-3p	Human	Plasma	Up-regulation
	miR-126-5p, 101, 199, 34	Rat	Lung	Down-regulation
	miR-223	Human	Plasma	Up-regulation
	miR-223-5p	Human	Blood	Up-regulation
	miR-223	Human	pPMVs	Down-regulation
	miR-223	Rat	Lung	Down-regulation
Hypertension	miR-320,	Rat	Aorta	Up-regulation
	26b, 21	Rat	Aorta	Down-regulation
	miR-208a	Rat	Plasma	Up-regulation
Hyperlipidemia	miR-122	Human	Plasma	Up-regulation
	miR-30c	Bream	Liver	Up-regulation
	miR-33a/b	Human	Plasma	Unchanged
	miR-223	Human	Serum	Up-regulation
Obesity and diabetes	miR-26a	Human	Liver	Down-regulation
	miR-26a, miR-126	Human	cMPs	Down-regulation
	miR-103/107	Mice	Liver	Up-regulation
	miR-143/145	Mice	Adipose Liver	Up-regulation
	miR-802	Human Mice	Liver	Up-regulation
Physical activity	miR-29	Rat	Heart	Up-regulation
	MiR-1, 133a	Human lateralis	Vastus	Down-regulation
	miR-222	Human	Serum	Up-regulation
	miR-222,146a	Human	Serum	Up-regulation

Buie, et al. Atherosclerosis. 22 September 2016

Biomarkers for CV Disease Risk Factors

- Cigarette Smoking
  - miR-126
  - miR-223
- Hypertension
  - miR-320
  - miR-208a/b
- Dyslipidemia
  - miR-122
  - miR-33
- Obesity/Sedentary Lifestyle
  - miR-26a
  - miR-222

Mipomersen: Lessons Learned from RNAi Technology

Mipomersen is a synthetic antisense RNA

Binds the ApoB mRNA

Prevents translation of the ApoB100 protein

nucleotides are linked with phosphorothioate linkages

capped with 2'-*O*-methoxyethyl- modified ribose

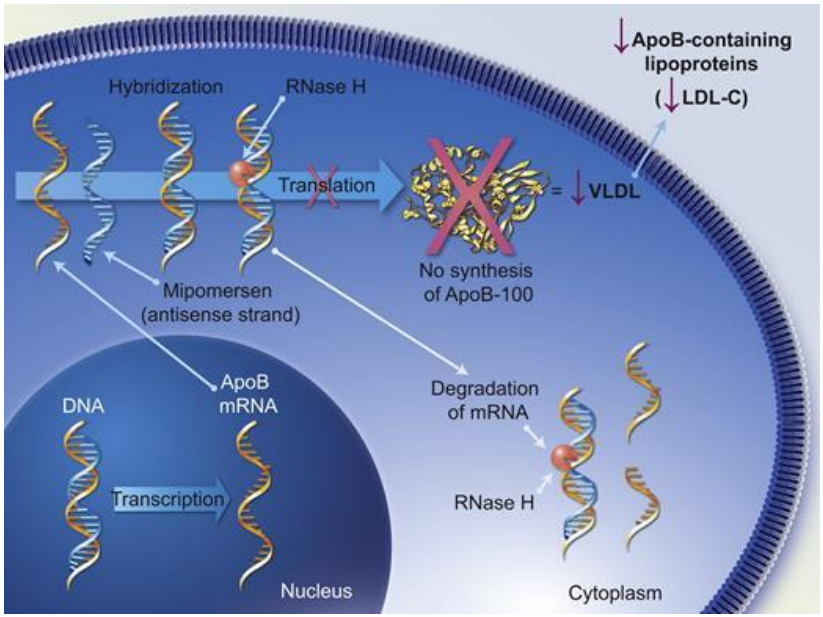
given as a weekly injection

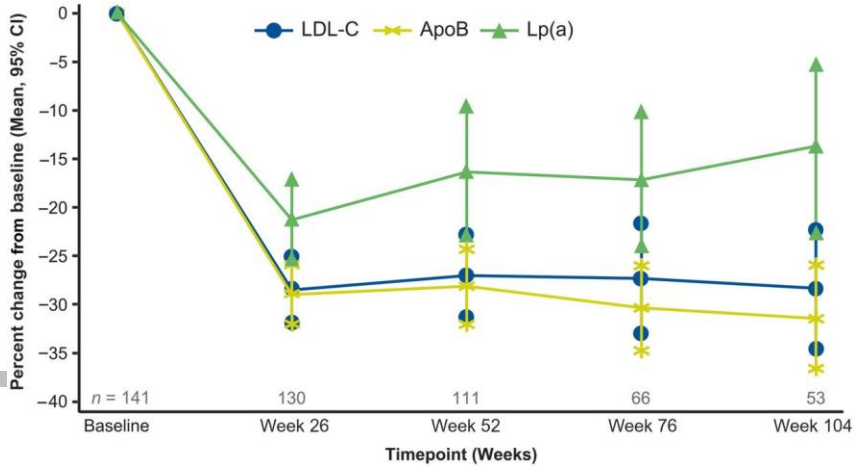
Effective for sustained reduction in LDL-C

Flu-like reactions are relatively common

Relatively rare serious effects are seen over 2 years

(some elevations in ALT, nonspecific GI side effects, etc)





miRNA Therapeutics for Cardiovascular Disease

anti-miR therapies exist in Phase II clinical trials for patients with hepatitis C

miR-based therapies are currently in pre-clinical and Phase 1 trials for multiple illnesses, primarily malignancies

Cardiovascular diseases currently being investigated include post-MI remodeling, chronic heart failure and peripheral arterial disease

COMPANY	COMPOUND	TARGET	ACTION	PRE-CLINICAL	PHASE I	PHASE II	PHASE III
Santaris Pharma	Miravirsen	miR-122	anti-miR	Hepatitis C virus			
EnGeneIC Ltd	MesomiR-1	miR-16	mimic	Mesothelioma			
miRagen Therapeutics	MRG-106	miR-155	anti-miR	Hematological Malignancies			
	MRG-107	miR-155	anti-miR	Amyotrophic Lateral Sclerosis			
	MRG-201	miR-29b	mimic	Cutaneous and Pulmonary Fibrosis			
	MGN-1374	miR-15/195	anti-miR	Post-MI Remodelling			
	MGN-4893	miR-451	anti-miR	RBC Disorders			
	MGN-9103	miR-208	anti-miR	Chronic Heart Failure			
	Unknown	miR-92a	anti-miR	Wound Healing and PAD			
MiRNA Therapeutics	MRX34	miR-34	mimic	Solid Tumors			
	MRX34	miR-34	mimic	Hematological Malignancies			
	MiR-Rxlet-7	Let-7	mimic	Lung Cancer			
	MiR-Rx-06	miR-16	mimic	Prostate Cancer			
	Unknown	miR-101	mimic	Cancer			
	Unknown	miR-215	mimic	Cancer			
Regulus Therapeutics	RG-012	miR-21	anti-miR	Alport Syndrome			
	RG-101	miR-122	anti-miR	Hepatitis C Virus			
	RG-125 (AZD4076)	mIR-103/107	anti-miR	NASH			
	Unknown	miR-10b	anti-miR	Glioblastoma			
	Unknown	miR-221	anti-miR	Hepatocellular Carcinoma			
	Unknown	miR-350	anti-miR	Neuroblastoma			

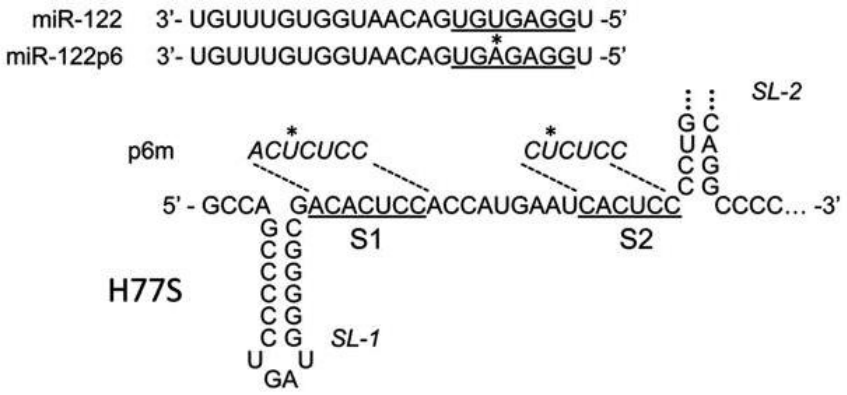
Mitchell, et al Pharmacology & Therapeutics (Sep 2016)

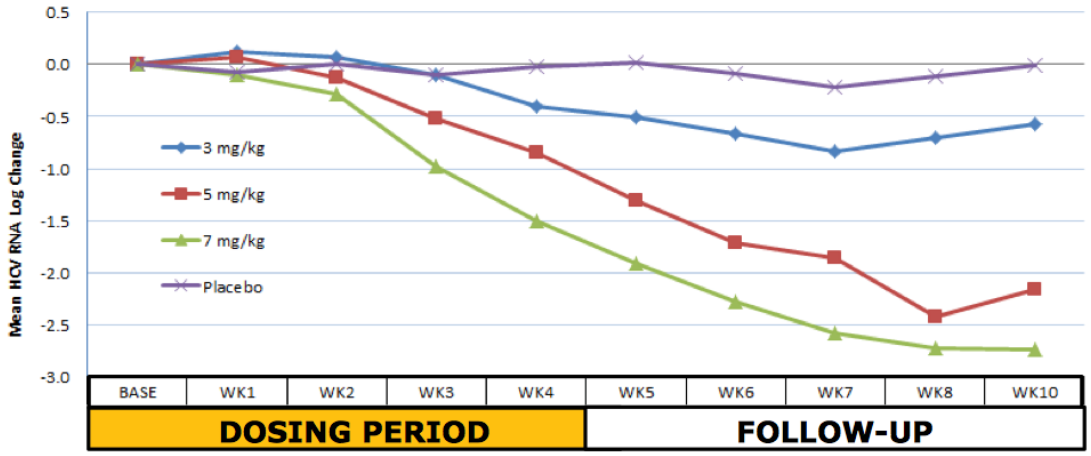
Miraversen: miRNA Therapy for Hepatitis C

Miraversen: an antisense modified oligonucleotide that inhibits miR-122

miR-122 binds HCV RNA and promotes translation

Inhibition of miR-122 reduced HCV viral load in initial trials with no significant side effects at 4 weeks of therapy





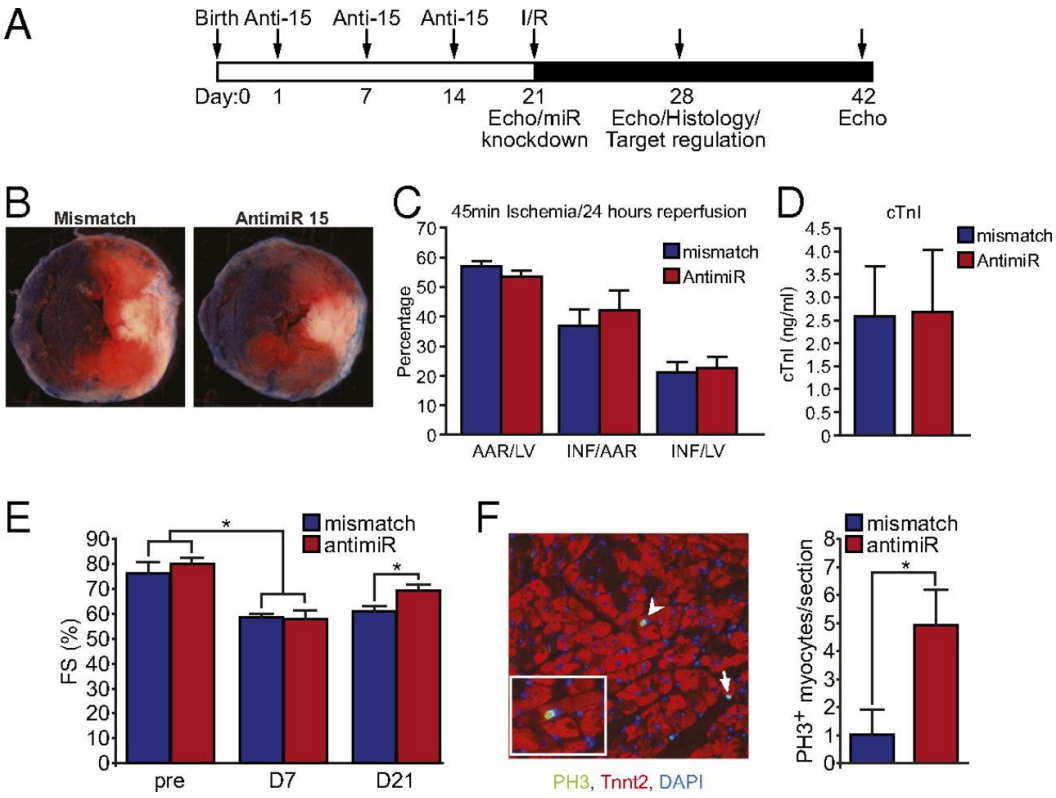
Proc Natl Acad Sci U S A. 2013 Jan 29; 110(5): 1881–1886.
Data courtesy of Santaris Pharmaceuticals, Inc

miR 15/195

MGN-1374

Inhibitor of miR 15/195

Preclinical animal trials show improvement in proliferation of cardiomyocytes post-infarction

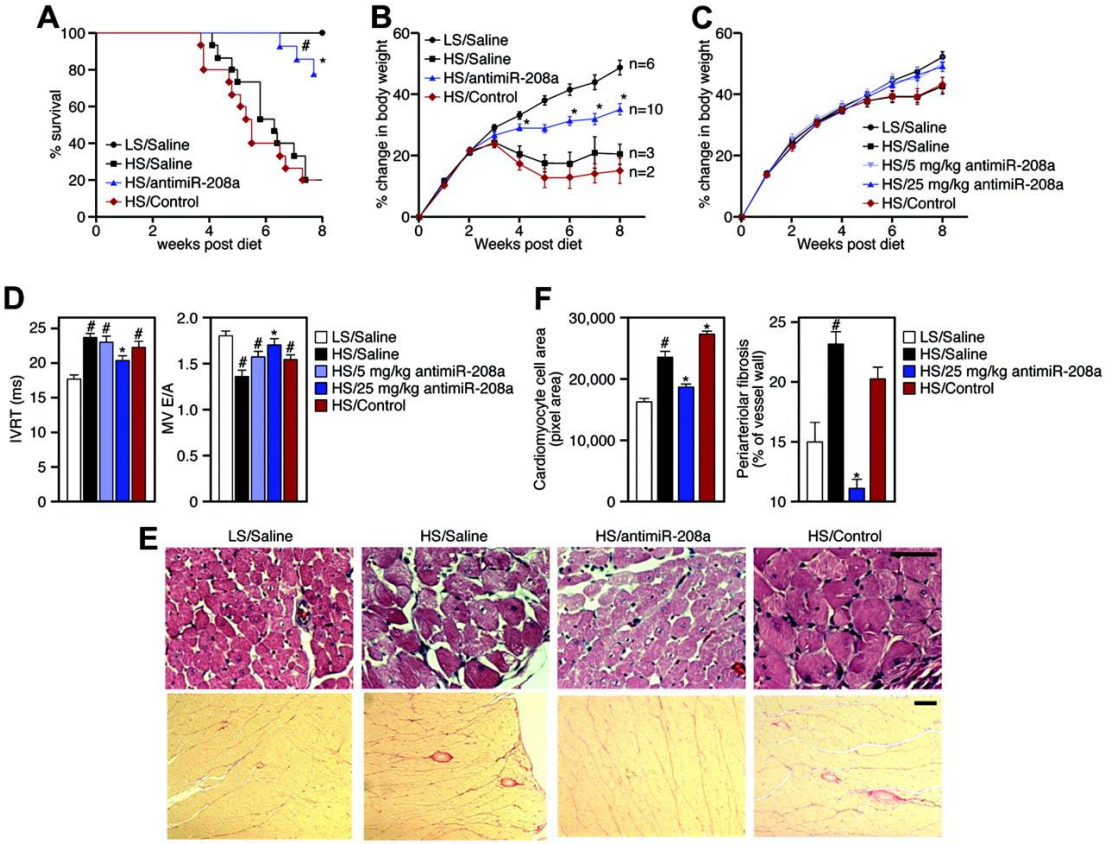


Porrello, et al. Proc Natl Acad Sci U S A, 110 (2013), pp. 187–192

miR208

MGN-9103 is an inhibitor of miR-208a

phosphorothiolated oligonucleotide



Montgomery, et al. Circulation, 124 (2011), pp. 1537–1547

Potential Side Effects to miR Therapy

miRs are promiscuous molecules

prolonged exposure to anti-miR or pro-miR therapy may lead to numerous side effects

miR therapy is in its infancy

Conclusions

miRNAs inhibit translation of multiple gene targets leading to various effects throughout multiple systems

Anti-miR therapy has been shown to be preliminarily effective in Phase 1-2 trials for limited indications (HCV)

The role of miR therapy in cardiovascular disease has yet to be clarified in humans, though a potential for therapeutic benefit exists based on animal trials

The technology exists to alter miR activity and may prove to be a staple of therapy in the future for chronic cardiovascular disease