miRNA Biomarkers

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Outline

Introduction

Circulating miRNAs as potential biomarkers for pain

- *Complex regional pain syndrome (CRPS) & rodent models*

Pharmacological intervention induced miRNA modulation

- *Ketamine treatment for CRPS & celecoxib in rodent model*

Source of circulating miRNAs

- *Exosomes*

Perspectives on possibilities . . . . .

Circulating microRNAs as biomarkers

MicroRNAs (miRNAs) are a class of small non-coding RNAs ~ 22 nucleotides

Aberrant miRNA expression and miRNA-mediated gene regulation observed in human diseases

Extracellular miRNAs in serum, plasma, saliva, and urine are associated with various pathological conditions

miRNA expression patterns capable of classifying tumors according to type and tissue of origin, poorly differentiated tumors

Circulating miRNAs are novel, noninvasive biomarkers amenable to clinical diagnosis in translational medicine

What attributes make circulating miRNAs feasible as biomarkers?

Abundant in circulation *(non-invasive)*

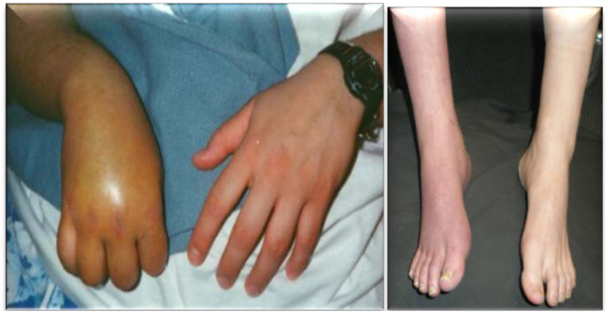
Can be measured by qPCR *(specific, sensitive, accurate)*

Stable *(robust, translatable)*

Associated with diseases *(predictive)*

The subjective nature of pain scoring and assessment in humans highlights the need for the development of objective measures to assess pain

miRNA modulation in complex regional pain syndrome



CRPS is a progressive neurological disease with sensory, autonomic and motor disturbances

Compared expression of ~750 miRNAs in whole blood from CRPS patients (n=40) and controls (n=20)

18 miRNAs were differentially expressed in CRPS patients

Three different groups emerged from miRNA profiling. One group was composed of 60% CRPS patients and contained no control subjects

miRNA profiling of whole blood can be a novel, clinically relevant approach for patient stratification

Scores recorded for different types of pain

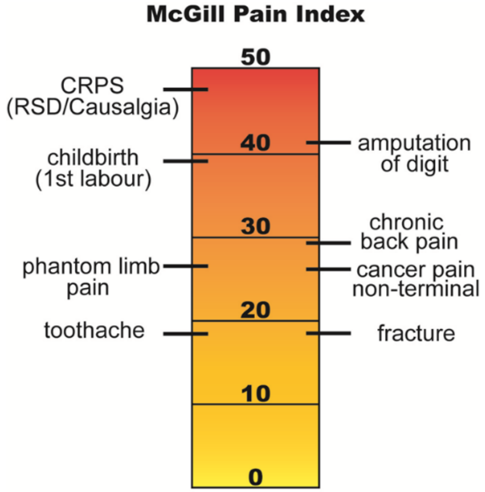


Figure adapted from Katz and Melzack (1999)

Orlova et al., 2011

Differentially expressed miRNAs and cytokines in CRPS

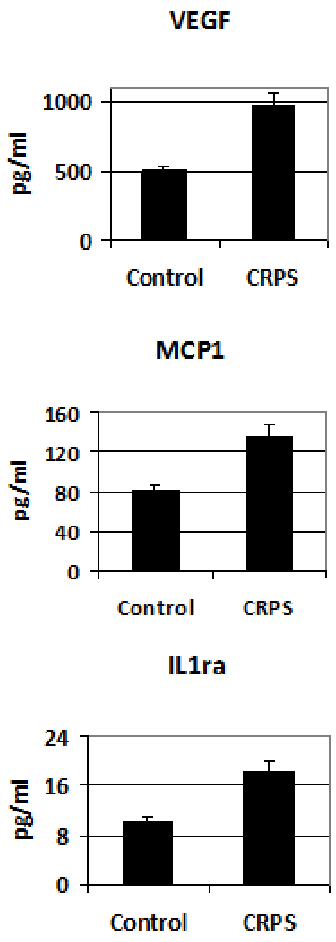
18 differentially expressed miRNAs

miRNA	*Fold change*	*p value*
hsa-miR-939	-4.59358	5.55E-06
hsa-miR-25#	-3.92328	1.09E-06
hsa-let-7c	-2.53502	2.07E-05
hsa-let-7a	-2.45923	0.002308
hsa-let-7b	-2.40344	5.49E-05
hsa-miR-320B	-2.0527	6.91E-06
hsa-miR-126	-2.02362	0.002469
hsa-miR-629.A	-1.71231	0.000653
hsa-miR-664	-1.54881	0.001487
hsa-miR-320	-1.44273	7.28E-05
hsa-miR-1285	-1.41594	0.003077
hsa-miR-625#	-1.33174	0.003542
hsa-miR-532-3p	-1.27226	0.001226
hsa-miR-181a-2#	-1.25927	0.000229
RNU48	1.348125	0.000391
hsa-miR-720	1.476853	0.003243
RNU44	1.854213	0.000904
hsa-miR-1201	2.14584	3.17E-05

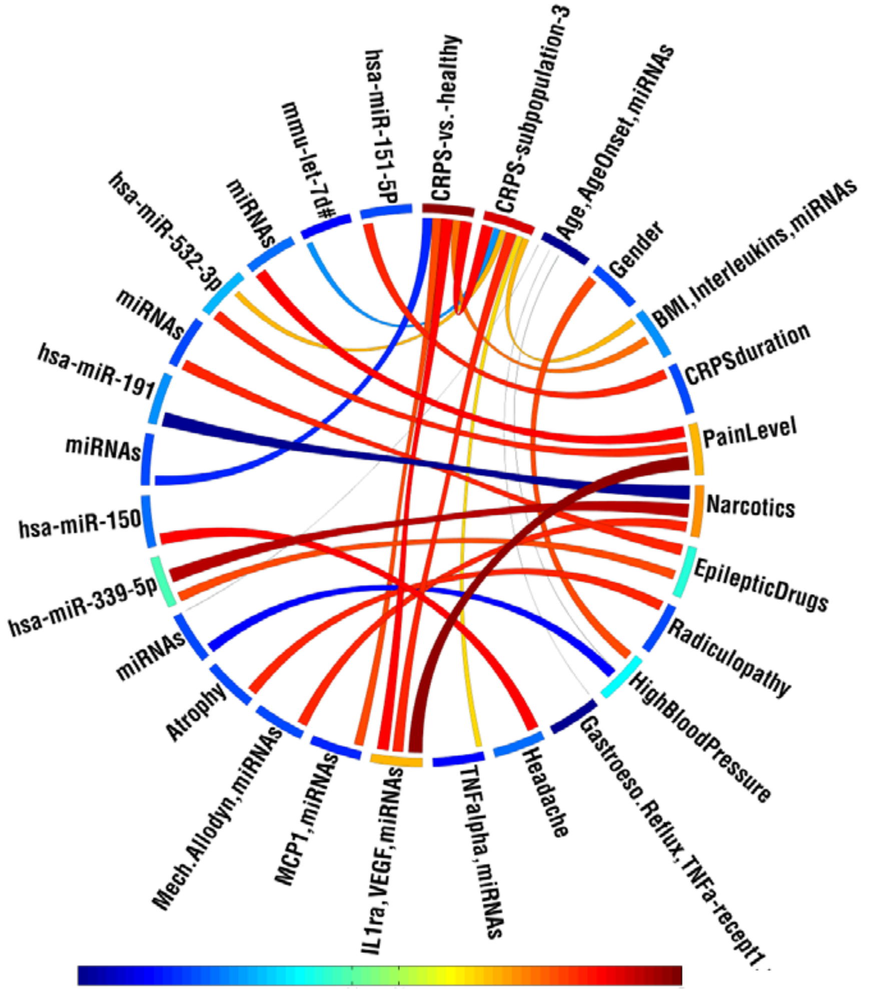
CRPS n=40 and control n=20

Orlova et al., 2011

VEGF, ILR1a and MCP1 significantly elevated



Circos diagram showing the correlation of selected parameters and miRNAs



negative correlation positive correlation

The nodes along the circle are colored by the total strength of correlation of the corresponding variable

Biomarkers – rodent models of pain

Bridging preclinical and clinical studies

Identify changes in miRNA expression in blood samples from rodent models of neuropathic and inflammatory pain

Identify miRNAs common to different models

Alternatively, different pain conditions/models may have unique profile

Species difference and miRNA signature (rats vs mice)

Models

Neuropathic pain

- Spared Nerve Injury (SNI) (mice) 4 weeks
- Spared Nerve Injury (SNI) (rats) 5 weeks
- Spinal Nerve Ligation (SNL) (rats) 5 weeks

Inflammatory pain (mice)

- Complete Freund’s Adjuvant (CFA)
- 3 days post-CFA
- 10 days post-CFA

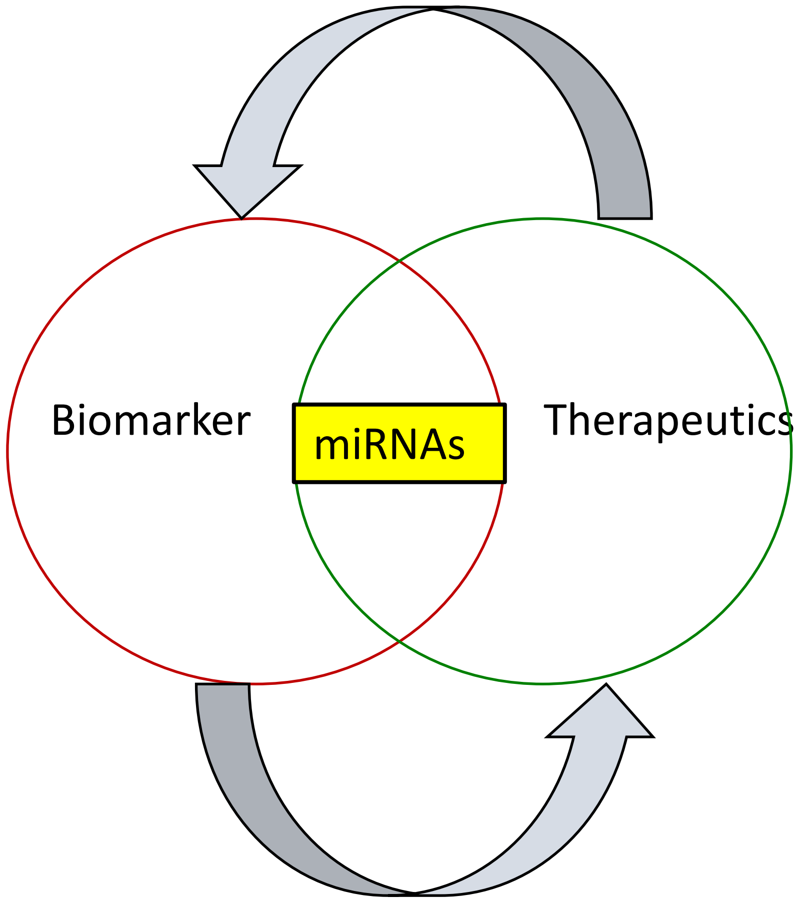
Qureshi et al., 2015

Circulating miRNAs in rodent models of pain - summary

- Differential expression of miRNAs in rodent blood
- miRNA signature in whole blood differed from those reported from spinal cord and DRG
- None of the differentially regulated miRNAs were common between mouse and rat SNI models, suggesting species-specific differences
- Temporal regulation of miRNA expression 3 and 10 days after CFA administration
- Two miRNAs in common with CRPS patients *(backward translation)*
- Computational miRNA target prediction and enrichment of predicted targets showed modulation of Wnt signaling

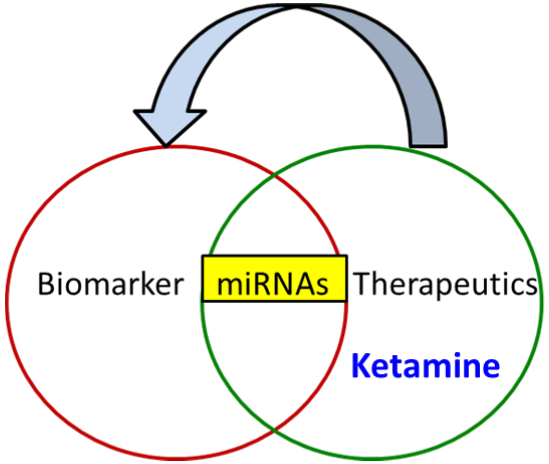
Qureshi et al., 2015

Pharmacological intervention induced miRNA modulation



Predictive validity of miRNA signature in determining the treatment outcome

Pharmacological intervention induced miRNA modulation – *CRPS patients*



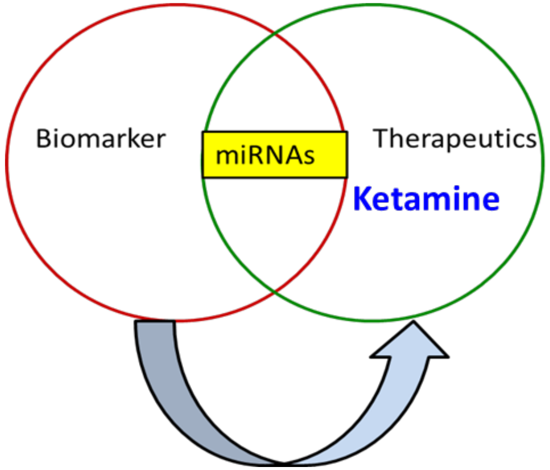
Ketamine treatment induced miRNA changes in CRPS patients (n=13)

Sub-anesthetic continuous intravenous administration for 5 days

Determine differential miRNA expression before and after treatment

Blood samples collected before and after treatment

Responders *(average pain score decreased by at least 50%)* & Poor responders



Can we distinguish responders vs poor responders prior to treatment?

Predictive validity of miRNA signature in determining the treatment outcome

Douglas et al., 2015

miRNA changes in ketamine responders and poor responders

Comparison of miRNA profiles before and after ketamine treatment showed

differential expression of 14 miRNAs in both responders and poor responders

miRNAs were not identical between the two groups

ketamine-induced molecular changes differ between responders and poor responders

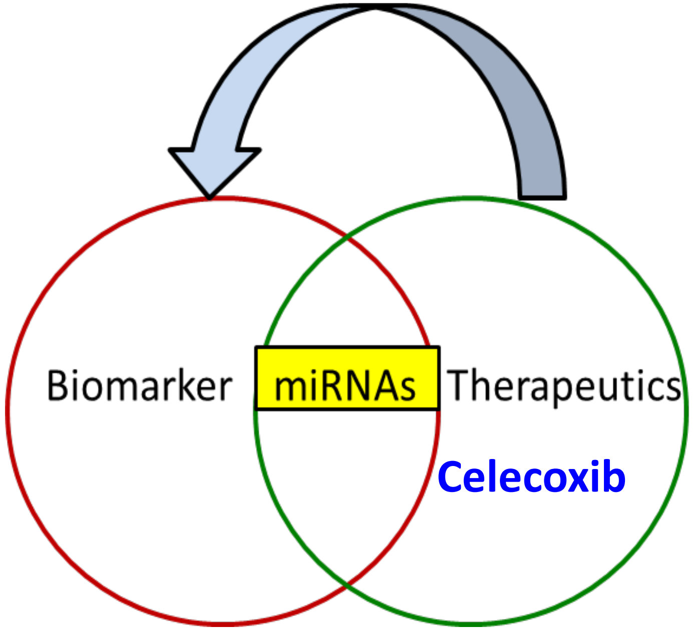
Comparison of miRNAs before treatment showed

differential expression of 33 miRNAs

miRNA signatures can be used as biomarkers for predicting treatment response

Douglas et al., 2015

Pharmacological intervention induced miRNA modulation - *rodents*



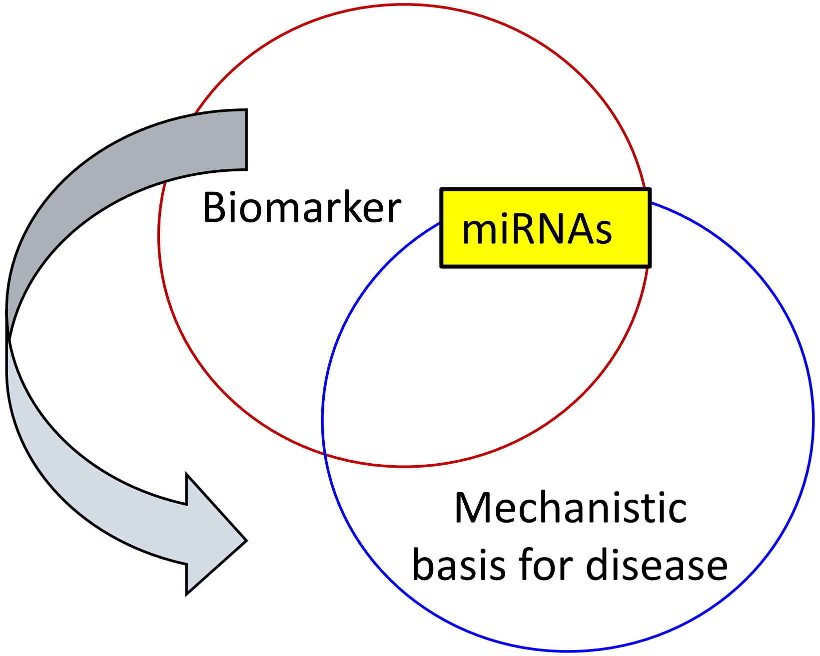
Inflammatory pain (mice); 10 days post CFA
Celecoxib administered post CFA injection (30 mg/kg for 7 days)

1) CFA + vehicle
2) CFA + Celecoxib
3) Saline + vehicle
4) Saline + Celecoxib

Across the six possible pairwise comparisons, 74 differentially expressed miRNAs were identified

Qureshi et al., 2015

Can differentially expressed miRNAs provide insight on the disease?



Biomarker

miRNAs

Mechanistic
basis for disease

Which genes are targeted by miR-939 and what is their relevance in pain and inflammation?

Functional consequences of hsa-miR-939 down regulation in CRPS patients

miRNA	**Fold change**	***p*-value**
hsa-miR-939	-4.6	5.55E-06

Genes targeted by miR-939 and their relevance in pain & inflammation

NOS2A	Nitric oxide synthase (inducible)
VEGFA	Vascular endothelial growth factor A
IL-6	Interleukin-6
NFκB2	Nuclear factor kappa beta

Modulating the levels of one miRNA capable of targeting several genes can amplify a pro-inflammatory signal transduction cascade

McDonald, et al., 2016

miRNA export from the cell and its stability

- Cells employ multiple distinct processes to release miRNA
  - Enclosed within membranous vesicles (exosomes, shedding vesicles and apoptotic bodies)
  - In association with high-density lipoprotein
  - Bound by RNA-binding proteins (Argonaute 2)

- Once in the extracellular space, miRNAs could be degraded by RNases, excreted, or taken up by other cells

- Exosomes deliver miRNAs to recipient cells with functional targeting capabilities

Exosomes – a novel method of intercellular communication

Small vesicles (30-100 nm) secreted by cells

Found in all body fluids

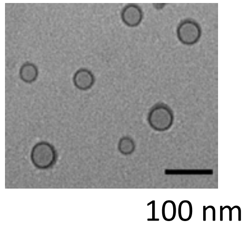
Carry miRNA, mRNA, proteins & lipids long distances

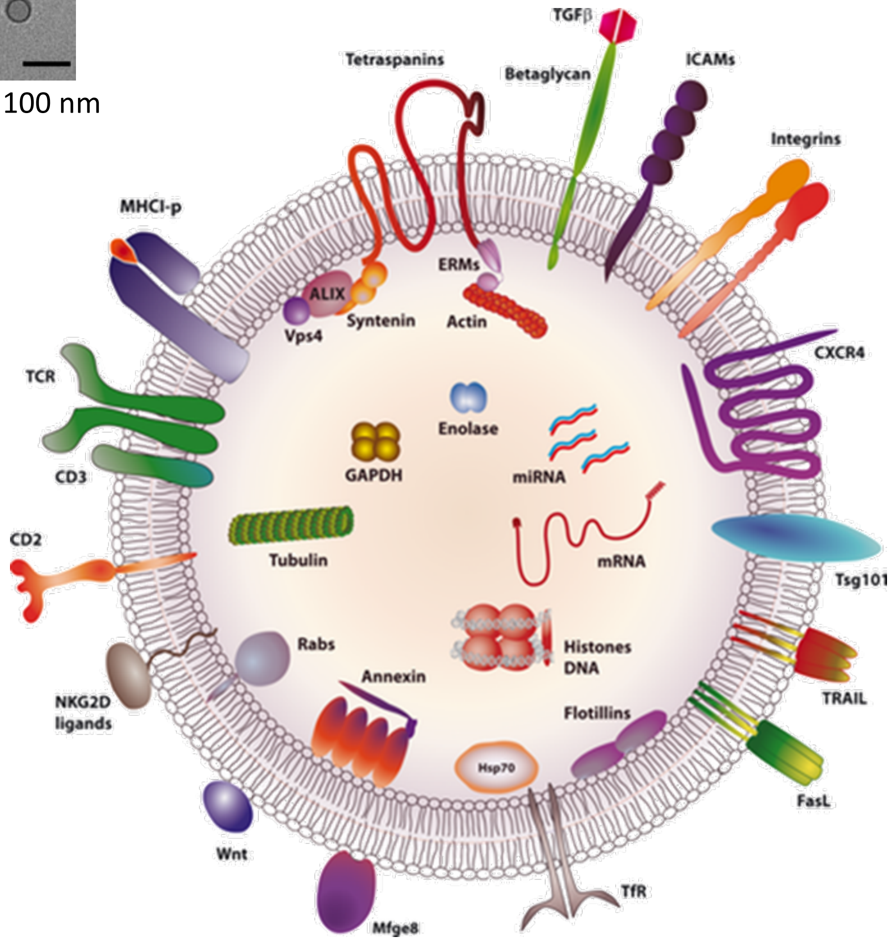
Mediate communication between cells

Exosomal composition depends on the physiological state of the parent cells

Potential biomarkers and therapeutics

Exosomes from mouse blood (*unpublished)*





Gutiérrez-Vázquez et al., 2013

miRNA profiling of exosomes from CRPS patients

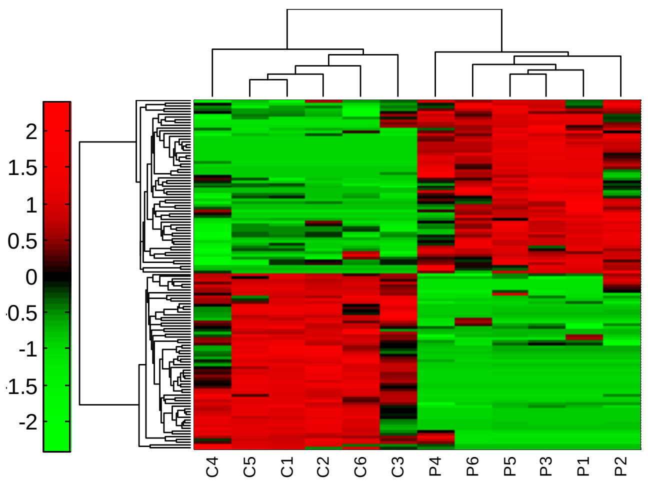
Exosomes purified by ultracentrifugation from the serum

6 control, 6 CRPS patients

127 of 503 detected miRNAs in human serum exosomes with significant alterations in CRPS patients

16 of the **18** miRNAs dysregulated in patients with CRPS from our previous study detected in exosomes

log10fc (red:high,black:avg,green:low)

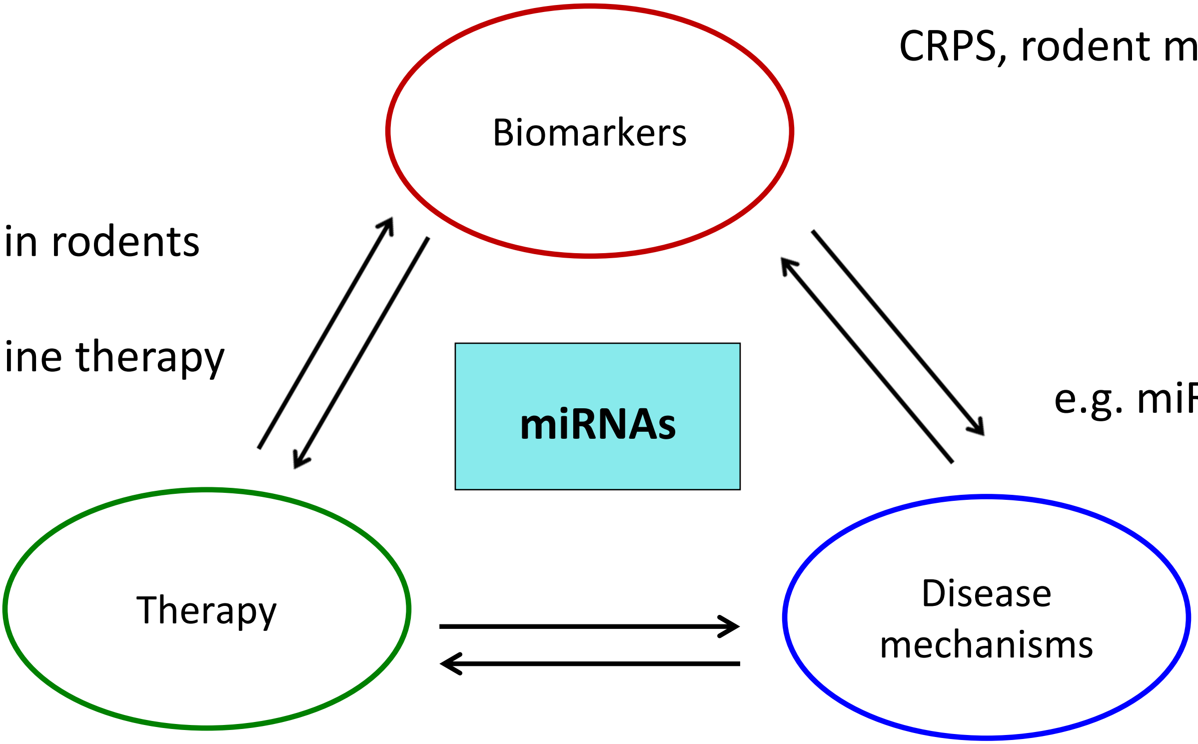


Benjamini-Hochberg false discovery rate correction to the results of a 2-tailed t-test

	Study 1	**Study 2**
Source	Whole blood	Exosomes
# of significantly altered miRNA	18	127
Sample procurement	Easy (blood in RNA stabilization solution, PAXgene blood RNA tubes)	Exosome purification is labor intensive (ultracentrifugation)
Exosomes are stable in serum and so retrospective studies are feasible		

McDonald, et al., 2014

Conclusions



Biomarkers

CRPS, rodent models of pain

Cox inhibitor in rodents

CRPS - Ketamine therapy

miRNAs

e.g. miR-939

Therapy

Disease
mechanisms

Responders vs poor responders in ketamine therapy
Ketamine pharmacokinetics

Exosome-mediated information transfer

Summary

Circulating miRNAs can be viable potential biomarkers, and links to previously unrecognized mechanisms of disease

Larger patient cohorts needed to reach firm conclusions regarding the diagnostic and prognostic power of extracellular miRNAs

Reasonable to assume that different painful conditions may have unique miRNA signature

Exosomal miRNA profiles showed larger number of significantly altered miRNAs

Identifying several miRNAs as biomarkers (molecular signature), rather than relying on one specific molecule or parameter, may increase the chances of successful treatment in an extremely heterogeneous group of patients suffering from pain

Circulating miRNAs can play a major role in finding the right molecules, targets, doses and patients

Some perspectives

Retrospective studies should be performed

Serum or plasma samples from failed clinical trials should be used to investigate miRNA signatures

Since the therapeutic outcome is known, patient stratification based on miRNAs will help determine if the drug is efficacious in a subset of patients with defined molecular signature

Biomarker discovery in clinical trials
miRNA profiles should be obtained starting from Phase I

Efficacy trials could be conducted in *defined patient groups*, guided by information obtained in preclinical and human volunteer models

Naïve control miRNA profiles should be made available in a centralized database along with clinical parameters

Guidelines to reduce variability

- Analytical variability (sample collection, storage, isolation methods, platform (qPCR, NGS), data normalization)
- Biological variability (duration of disease, sex, comorbidities, medications)

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