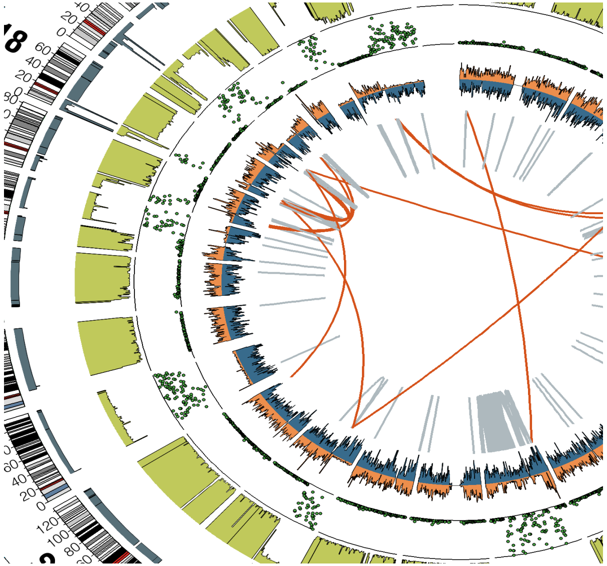

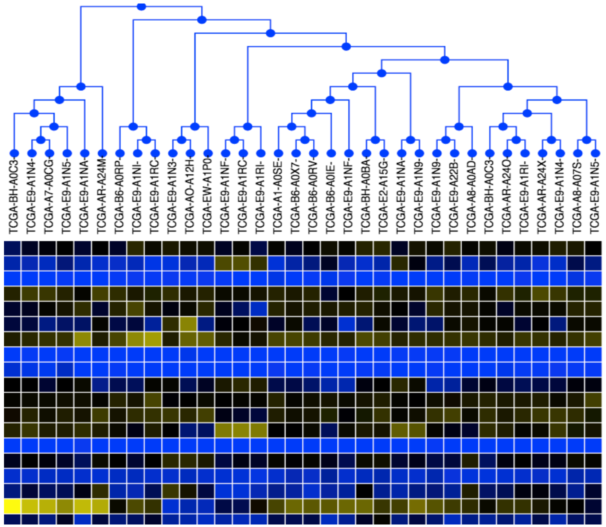

Applying Sequencing Technologies for Extracellular RNA Biomarker Discovery

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CENTER FOR
CANCER
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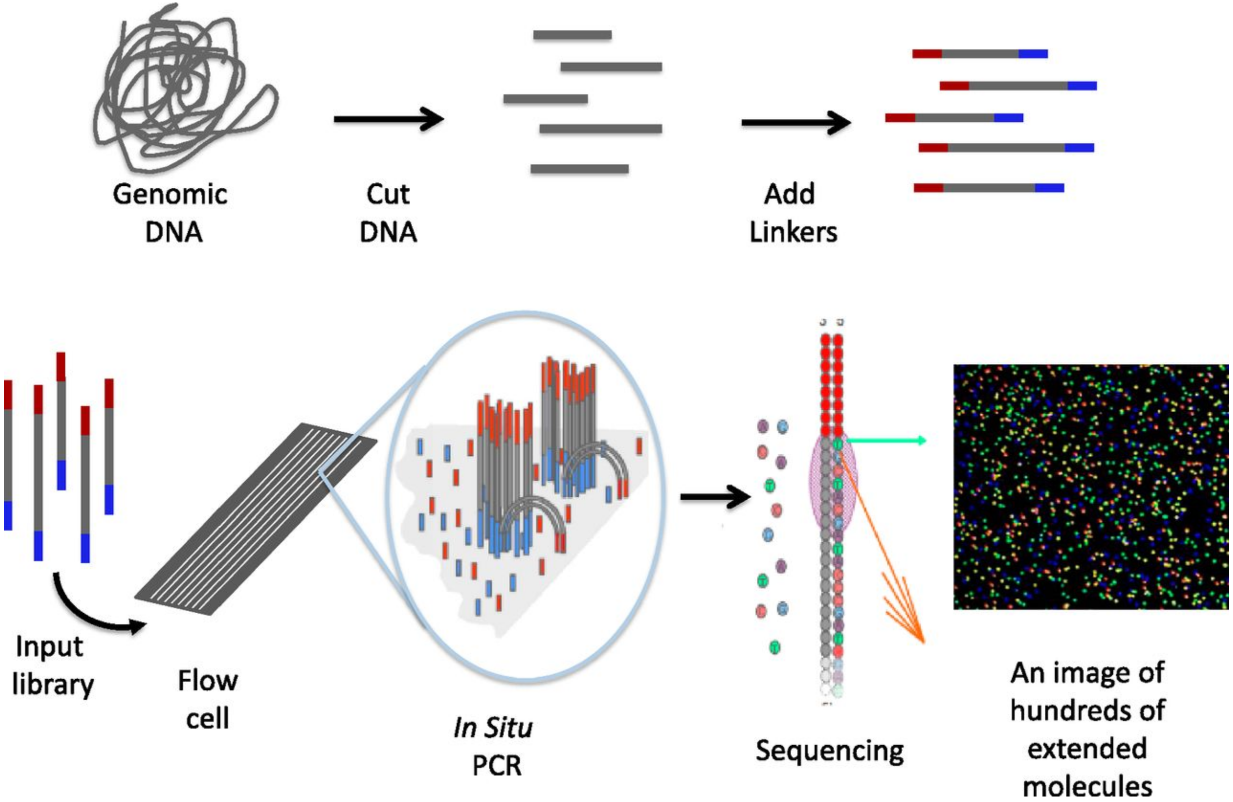
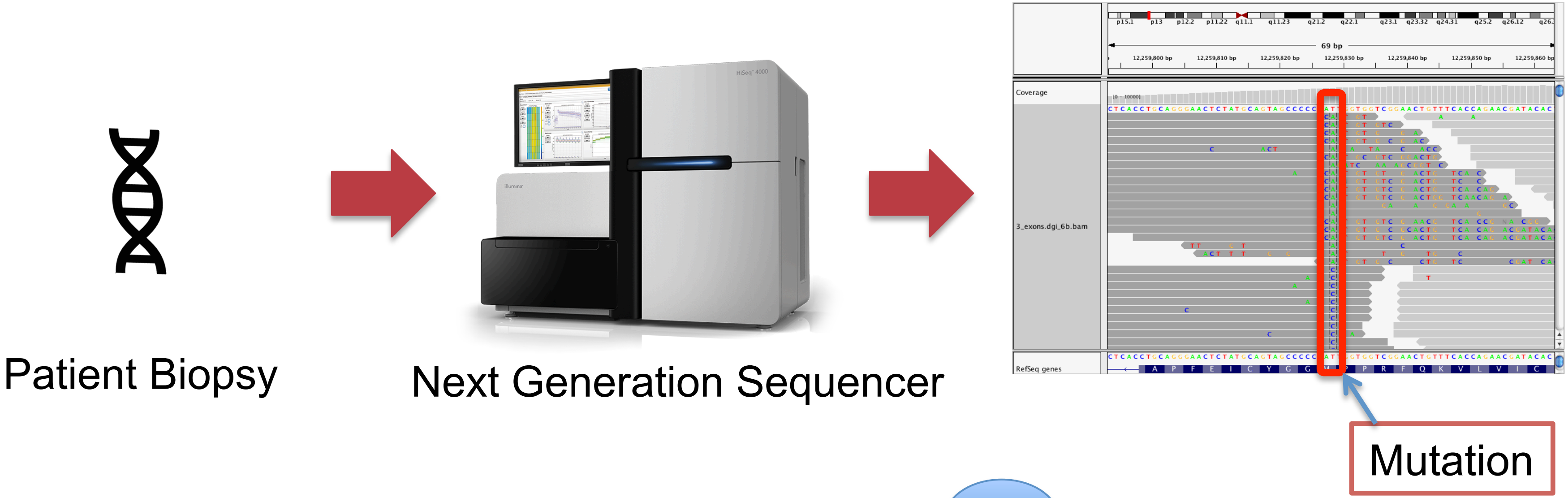
BIOLOGY
DANA-FARBER CANCER INSTITUTE

Massively Parallel Sequencing
(a.k.a Next Generation Sequencing)

Illumina HiSeq



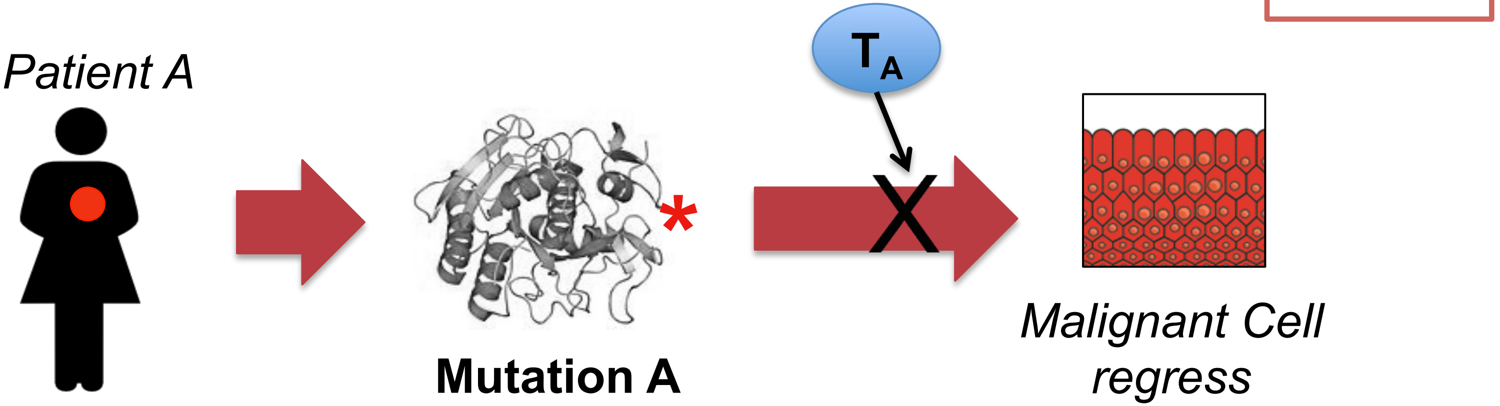
Treatment by Cancer Genomes



Patient Biopsy

Next Generation Sequencer

Mutation



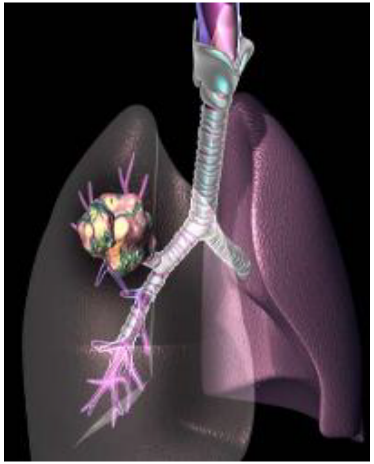
Patient A

Mutation A

Malignant Cell regress

Biomarker Drives Precision Medicine and Targeted Therapy

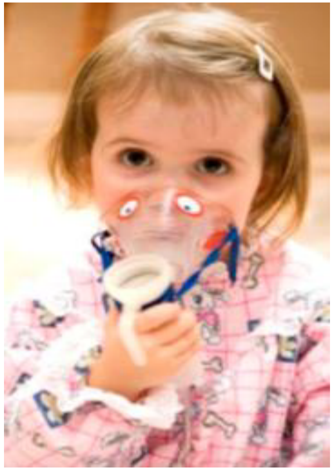
Her-2+
(Herceptin)
(Perjeta)



EML4-ALK
(Xalkori)

K-ras
(Erbitux)
(Vectibix)

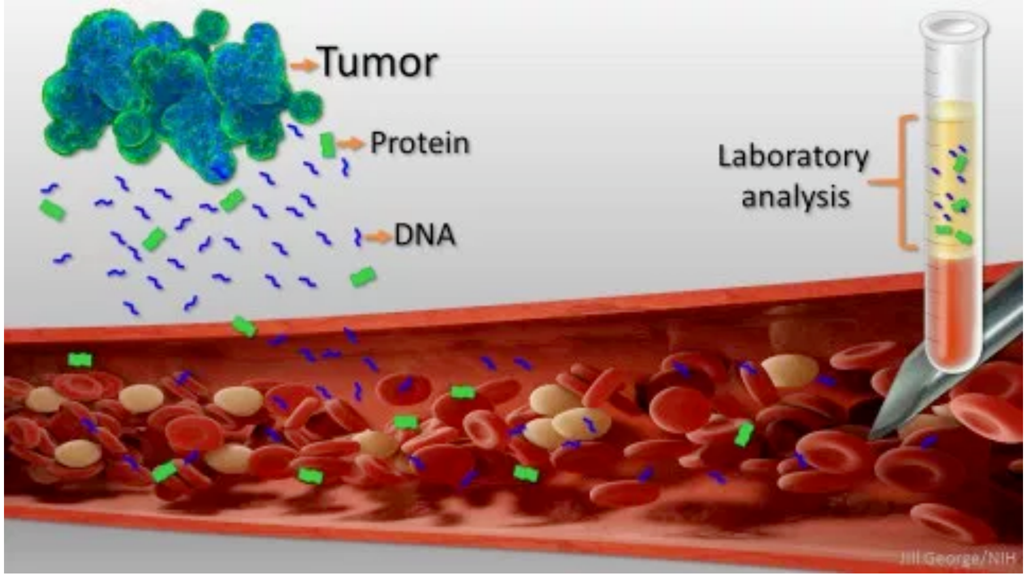
BRAF-V600
(Zelboraf)



CFTR-G551
(Kalydeco)

Companion Diagnostics: the Right Rx for the Right Disease (Subtype)

Liquid Biopsy Shows Early Promise in Detecting Cancer



Standard Biopsy: Time intensive procedure, localized sampling of tissue, some pain/ risk, Invasive, not easily obtained, late disease stage

Liquid Biopsy: Quick, comprehensive tissue profile, easily obtained, minimal pain/risk, minimally invasive, early detection

https://directorsblog.nih.gov/2018/01/30/new-liquid-biopsy-shows-early-promise-in-detecting-cancer/

Innovation in Cardiovascular Disease Remains a Challenge

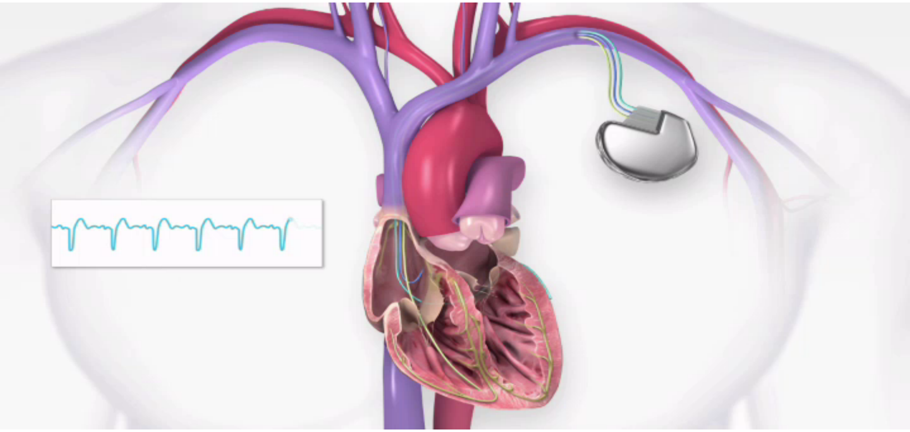
Clinical Need

- *17.3 million deaths per year globally due to CV disease*
- *CV disease remains the largest source of healthcare burden with $200bn in costs in the US alone*
- *Cardiac Resynchronization Therapy (CRT) alone is estimated to waste >$7bn in EU alone due to non-responders*

Innovation Challenges

- *<u>Inability to stratify patients</u> most likely to benefit from therapies have limited improvements in CV outcomes*
- *Newer cardiovascular drugs (PSCK-9 inhibitors, immunomodulators) face challenges with adoption given variable clinical benefit to heterogeneous populations*

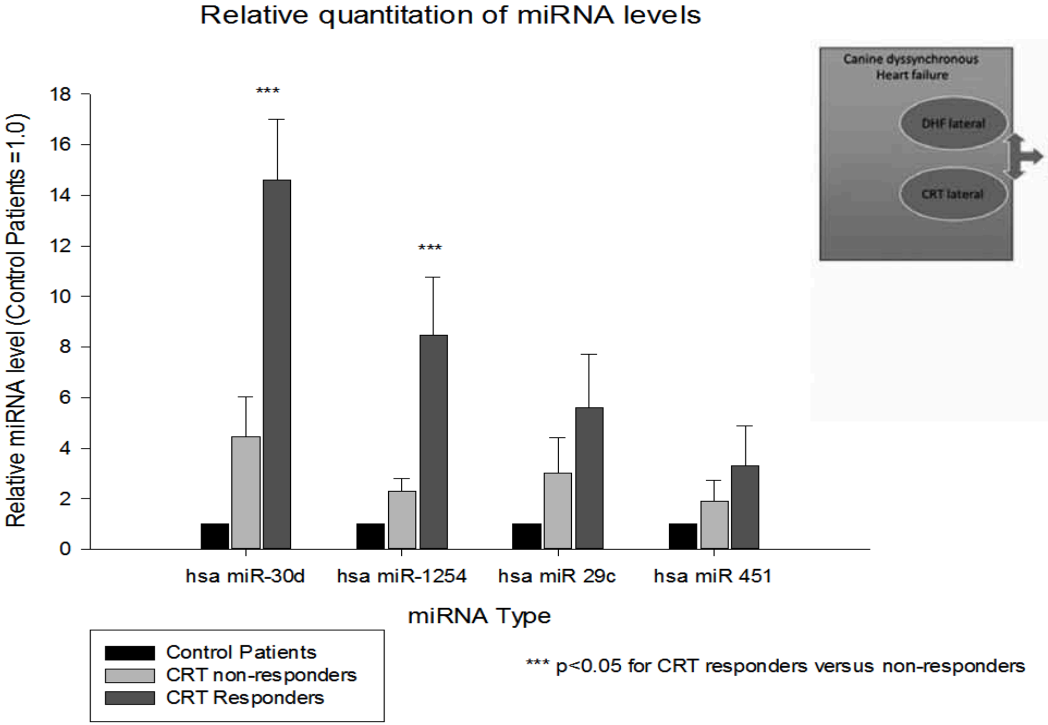
Patients Response differently to Cardiac Resynchronization Therapy (CRT)



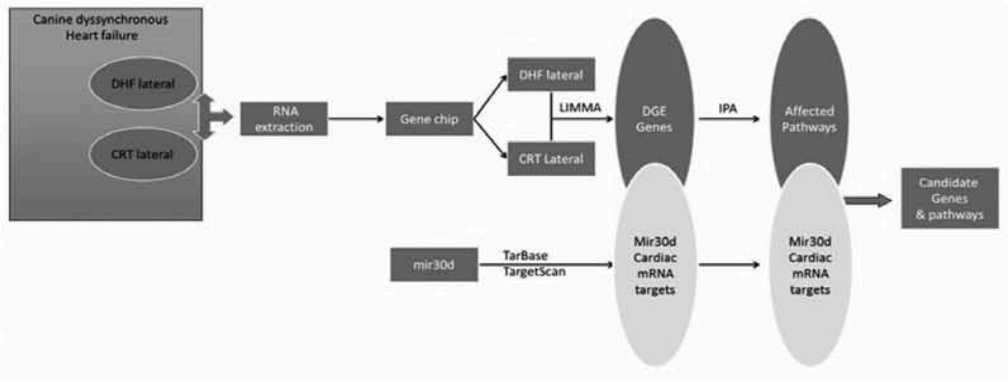
- Cardiac resynchronization therapy (CRT) treats electric dyssynchrony in heart failure patients
- Mitigates progressive decline in left ventricular function and poor prognosis in patients with heart failure
- >30% of the patients treated with CRT do not derived clinical benefits

http://watchlearnlive.heart.org/

Circulating MicroRNA-30d is associated with Response to Cardiac Resynchronization Therapy (CRT) in Heart Failure


Relative quantitation of miRNA levels

*** $p<0.05$ for CRT responders versus non-responders

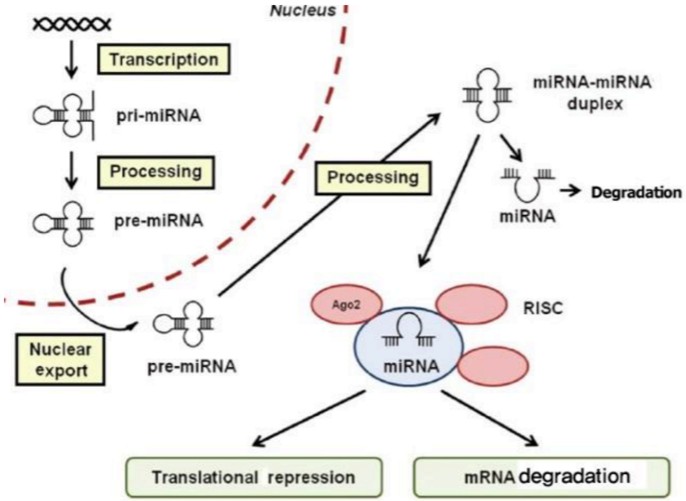


Found MIR30d level within patient plasma is associated with CRT treatment outcome in heart failure patients

Melman, et al Circulation (2015)

Regulatory Extracellular miRNA as Biomarkers

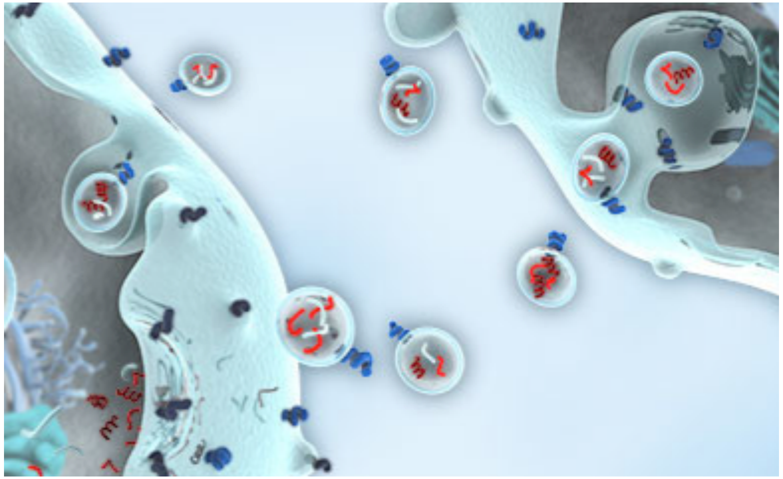
miRNA Regulate Gene Expression



<u>‘*Eavesdrop*’ inter-tissue communication</u>

- miRNA (~20bp) affects intercellular physiological process by regulating gene expression
- Measuring exRNA in blood intercepts communication between tissues in response to <u>current</u> body condition and disease state
- Could provides temporal prognostic information for treatment outcome

NGS as a tool for Extracellular microRNA Biomarker discovery



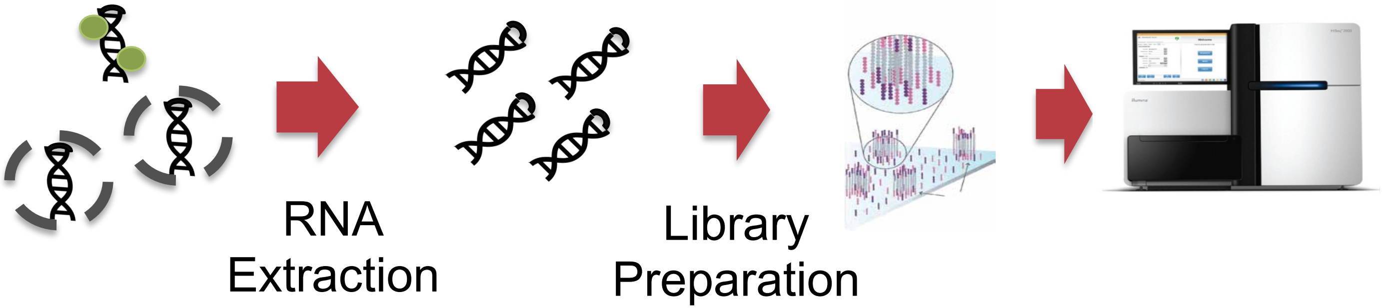
Benefits

- High sensitivity to wide microRNA expression range
- Sequence data contain high amount of information

Challenges

- Limited knowledge of RNA characteristic within extracellular space
- Low quantity and difficult to isolate
- No established analytical standard for exRNASeq expression data

Evaluate ExRNA data reproducibility



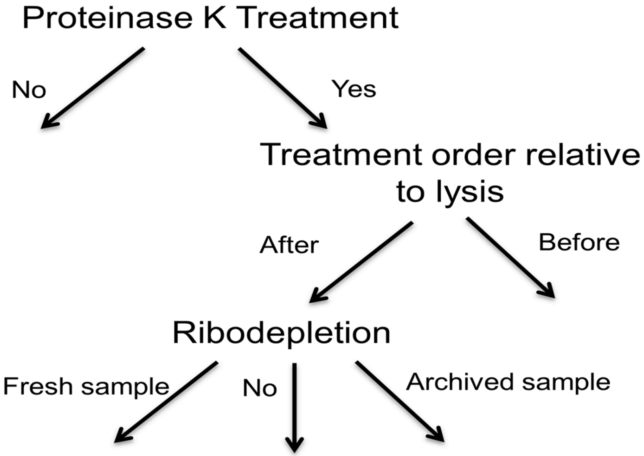
RNA Extraction

Library Preparation

Overall Strategy

- RNA extraction and library preparation were optimized iteratively to increase yield
- Plasma sRNASeq was performed on 3 healthy subjects with duplicates
- Compare identified miRNA levels with published plasma miRNA data

RNA Extraction Optimization



Proteinase K Treatment

No

Yes

Treatment order relative to lysis

After

Before

Ribodepletion

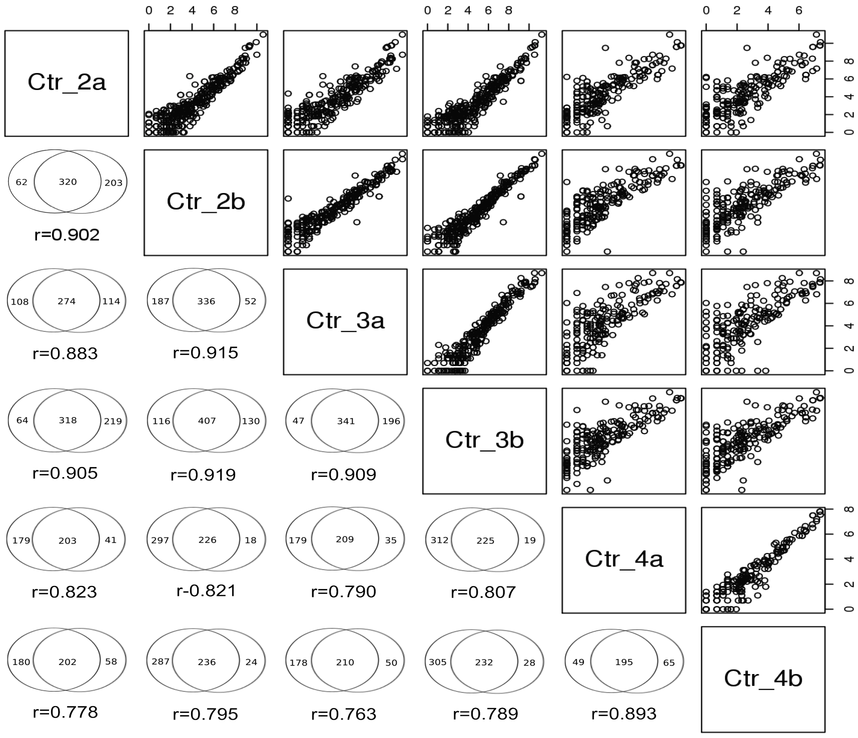
Fresh sample

No

Archived sample

Danielson, et al, PLoS One (2017)

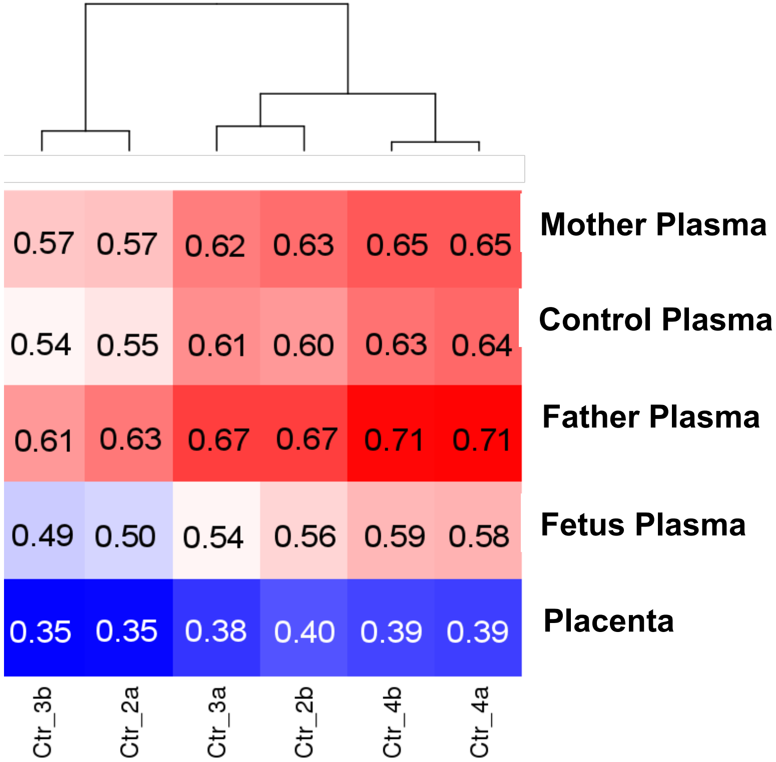
Intra/Inter Samples miRNA Expression Correlation



Intra sample: r≥0.9
Inter sample: r>0.75

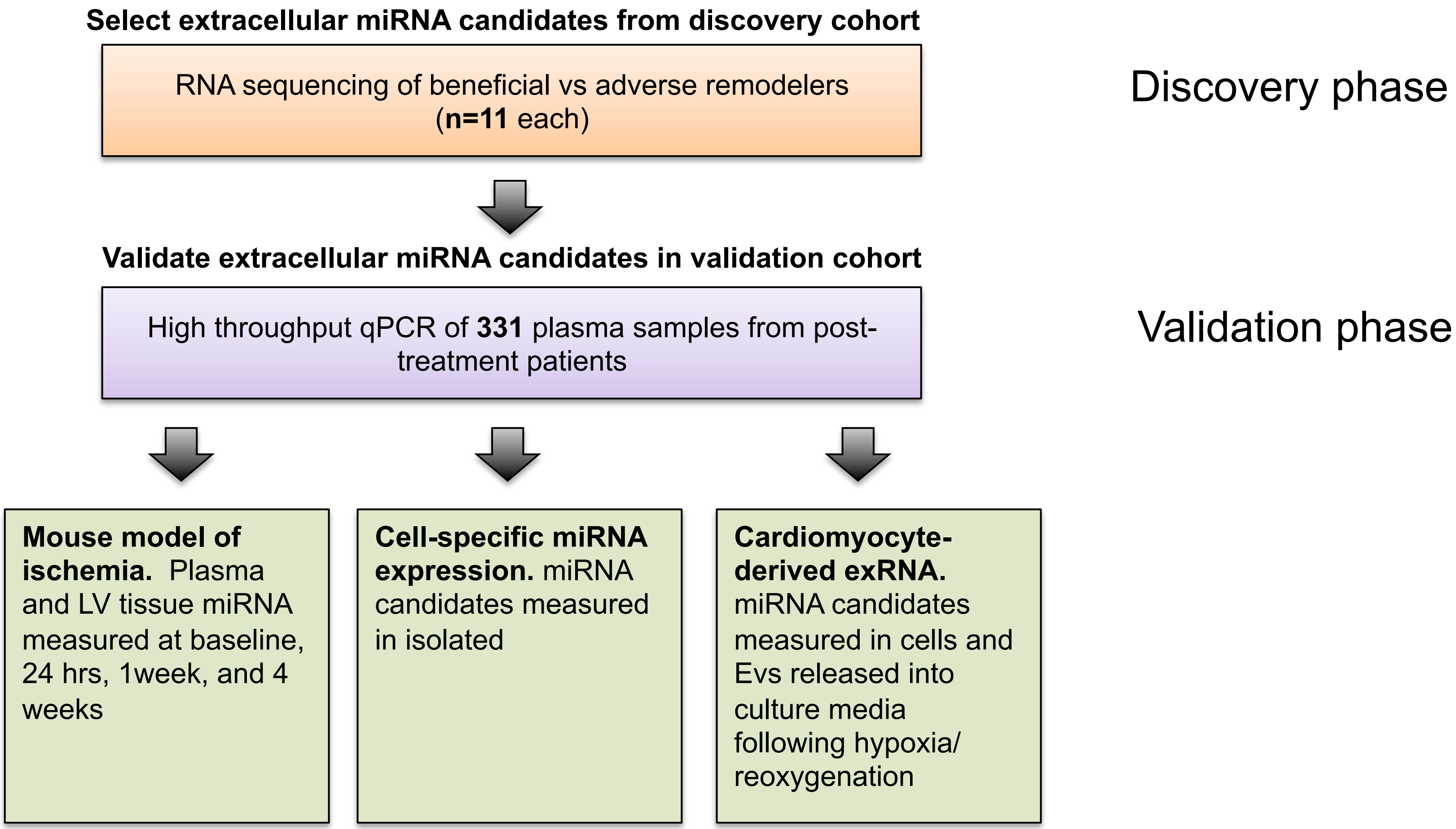
Comprehensive profiling of circulating microRNA via small RNA sequencing of cDNA libraries reveals biomarker potential and limitations

Zev Williams[a,b,c,d,1,2,3], Iddo Z. Ben-Dov[b,c,2,3], Rony Elias[a], Aleksandra Mihailovic[b,c], Miguel Brown[b,c], Zev Rosenwaks[a], and Thomas Tuschl[b,c,3]



	Ctr_3b	Ctr_2a	Ctr_3a	Ctr_2b	Ctr_4b	Ctr_4a
Mother Plasma	0.57	0.57	0.62	0.63	0.65	0.65
Control Plasma	0.54	0.55	0.61	0.60	0.63	0.64
Father Plasma	0.61	0.63	0.67	0.67	0.71	0.71
Fetus Plasma	0.49	0.50	0.54	0.56	0.59	0.58
Placenta	0.35	0.35	0.38	0.40	0.39	0.39

ExRNA Biomarker Discovery for CRT prognosis prediction – experimental set up



Select extracellular miRNA candidates from discovery cohort

RNA sequencing of beneficial vs adverse remodelers (**n=11** each)

Discovery phase

Validate extracellular miRNA candidates in validation cohort

High throughput qPCR of **331** plasma samples from post-treatment patients

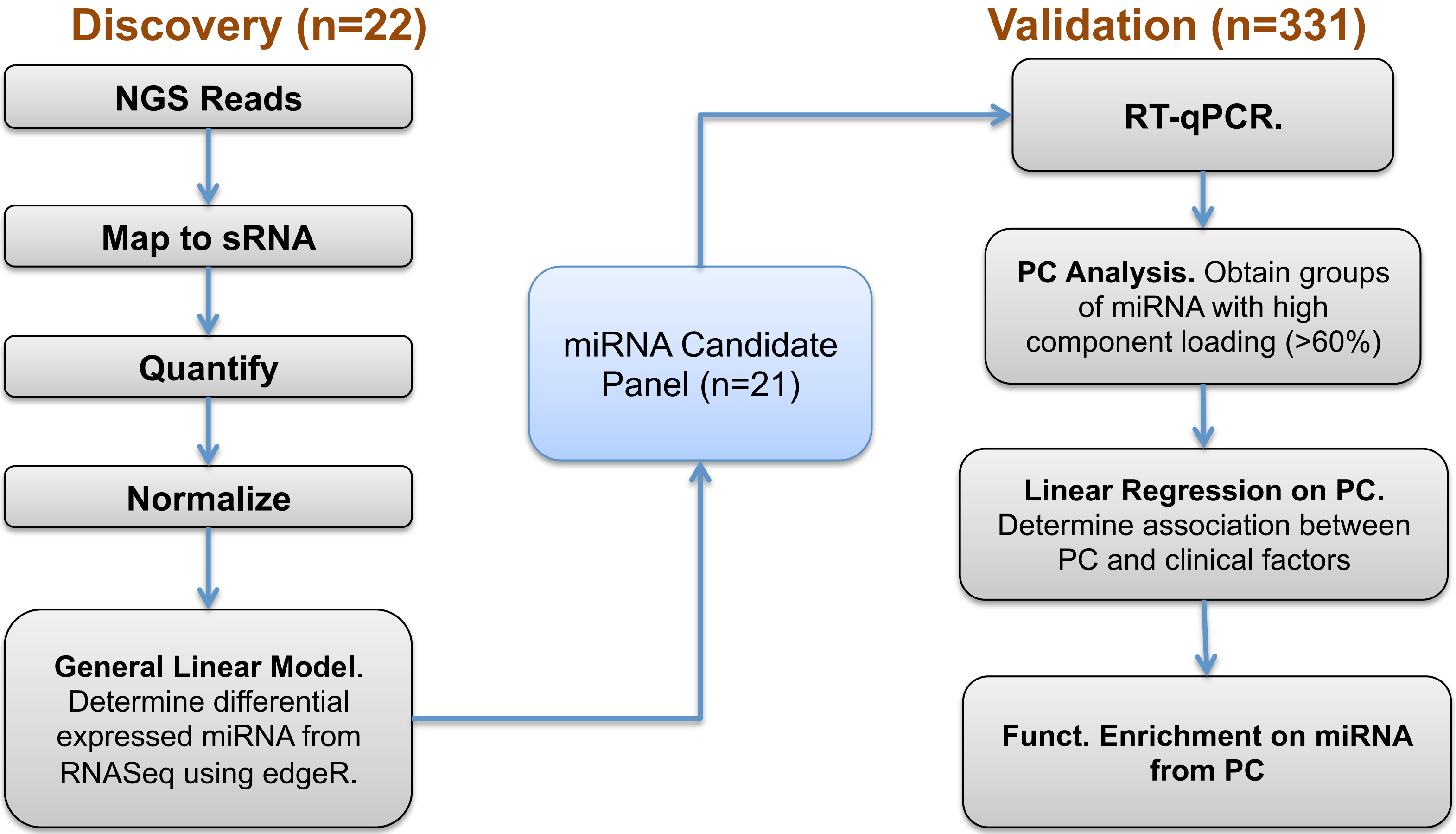
Validation phase

Mouse model of ischemia. Plasma and LV tissue miRNA measured at baseline, 24 hrs, 1week, and 4 weeks

Cell-specific miRNA expression. miRNA candidates measured in isolated

Cardiomyocyte-derived exRNA. miRNA candidates measured in cells and Evs released into culture media following hypoxia/ reoxygenation

ExRNA Biomarker Discovery for CRT prognosis prediction – Analysis Strategy



Discovery (n=22)

NGS Reads

Map to sRNA

Quantify

Normalize

General Linear Model. Determine differential expressed miRNA from RNASeq using edgeR.

miRNA Candidate Panel (n=21)

Validation (n=331)

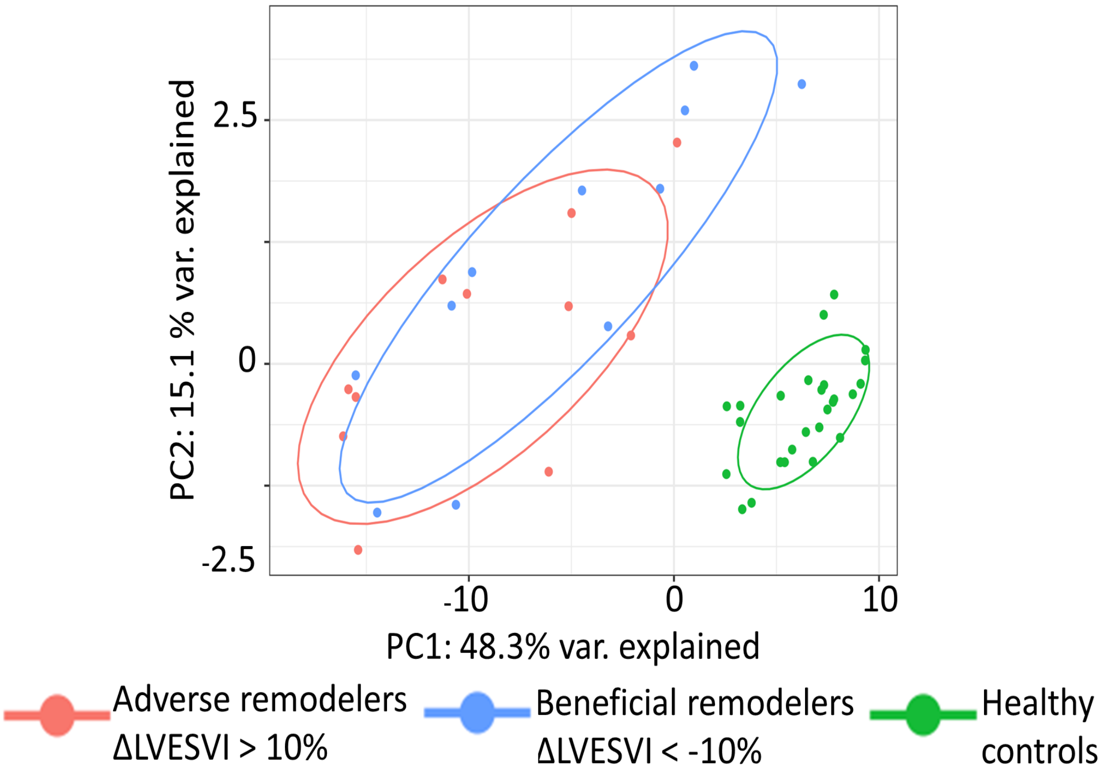
RT-qPCR.

PC Analysis. Obtain groups of miRNA with high component loading (>60%)

Linear Regression on PC. Determine association between PC and clinical factors

Funct. Enrichment on miRNA from PC

Principle Component Analysis on the miRNA candidates

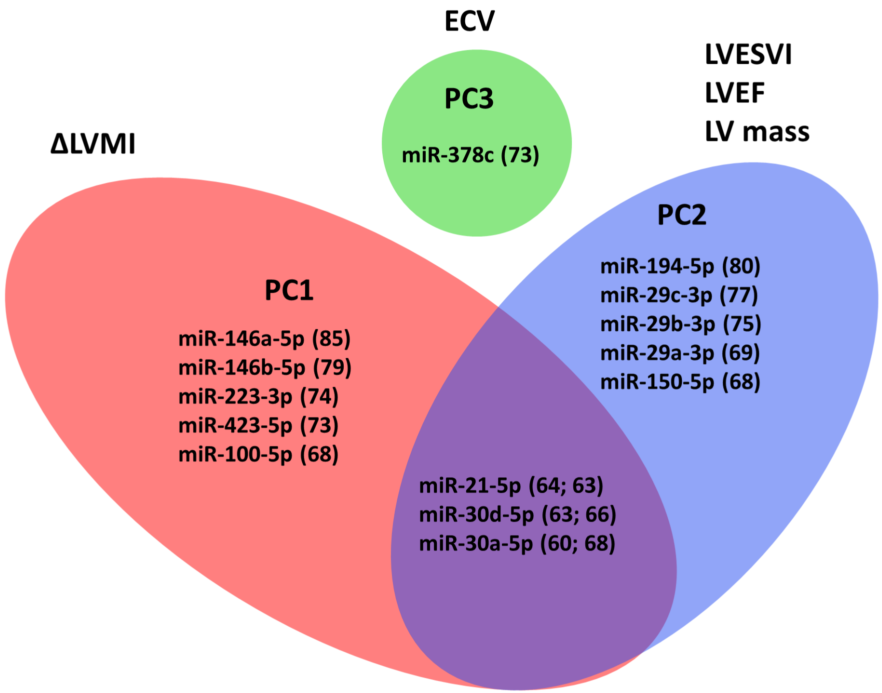


- Small separation between adverse and beneficial remodelers based on exRNA-Seq data

- Likely due to small sample size and variable sample quality

- 33 miRNA candidate panel was used with 12 known miRNA added

miRNA with high PC loadings are associated with clinical factors and pathways



ECV

PC3

miR-378c (73)

ΔLVMI

PC1

miR-146a-5p (85)
miR-146b-5p (79)
miR-223-3p (74)
miR-423-5p (73)
miR-100-5p (68)

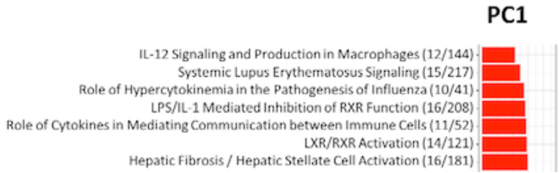
LVESVI
LVEF
LV mass

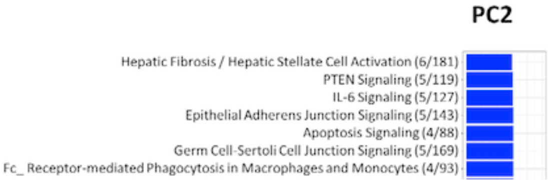
PC2

miR-194-5p (80)
miR-29c-3p (77)
miR-29b-3p (75)
miR-29a-3p (69)
miR-150-5p (68)

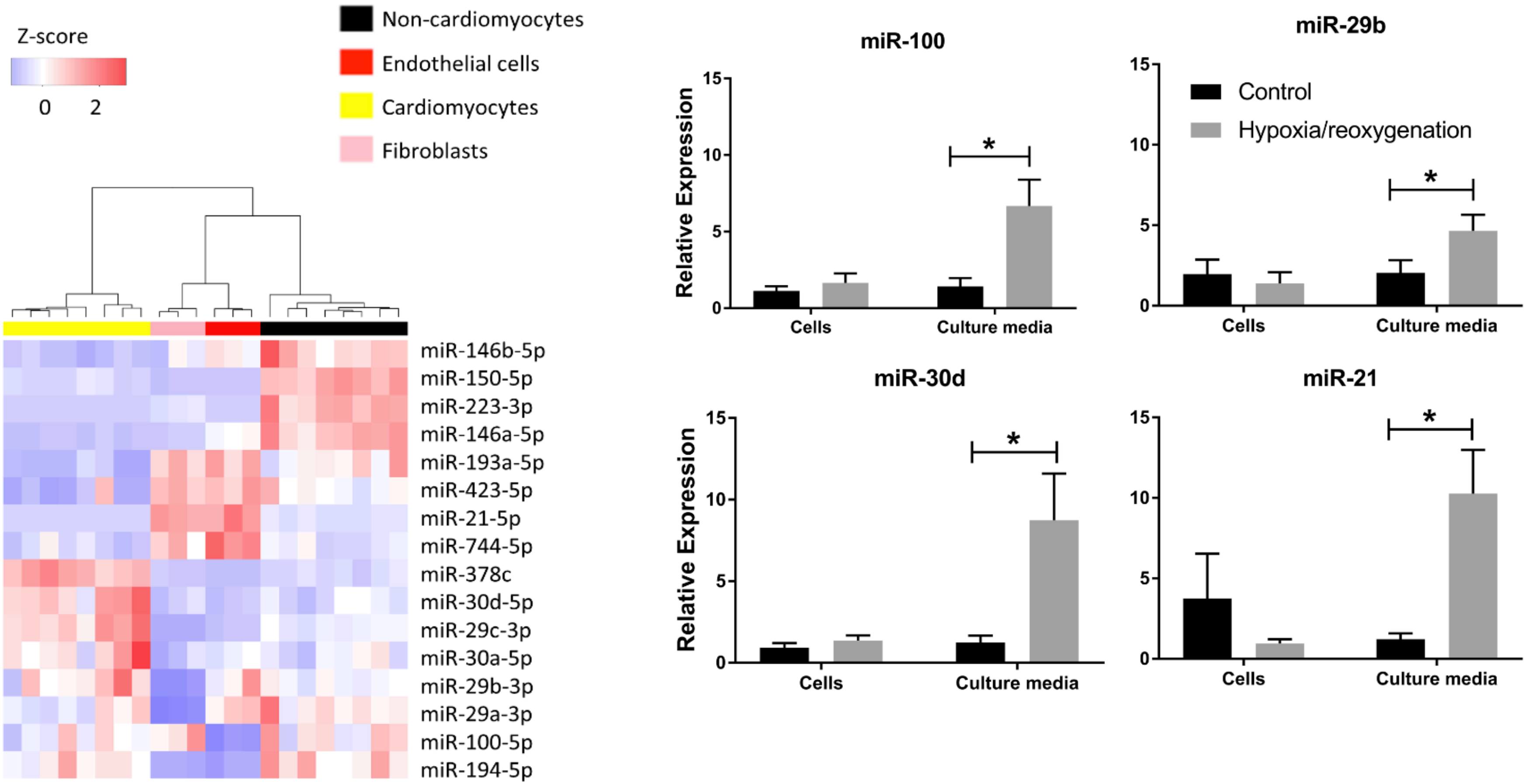
miR-21-5p (64; 63)
miR-30d-5p (63; 66)
miR-30a-5p (60; 68)

- Vast majority of these patients exhibiting relatively favorable LVRm
- PC analysis compress miRNA into sets to compare against CMR measurements
- miRNA show strong theme of inflammatory and fibrotic pathways





Candidate miRNA expression form distinct cluster by cell type and hypoxia state



Summary

- Extracellular RNA content reflects disease biology
- Advance in NGS technology enables comprehensive survey of extracellular RNA from biofluid
- Extract RNA sequence information from archived biofluid remains challenging
- Extracellular RNA biomarker offers the possibility to track dynamic disease state of complex CV pathophysiology and population heterogeneity

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