# Extracellular RNA

Cristina Sisu

Journal Club

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# Literature

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## Extracellular small RNAs: what, where, why?

**Anna M. Hoy and Amy H. Buck**[1]
Centre for Immunity, Infection and Evolution, Ashworth Laboratories, King's Buildings, University of Edi

*7248–7259 Nucleic Acids Research, 2010, Vol. 38, No. 20* *Published online 7 July 2010*
*doi:10.1093/nar/gkq601*

## Export of microRNAs and microRNA-protective protein by mammalian cells

**Kai Wang, Shile Zhang, Jessica Weber, David Baxter and David J. Galas**[*,†]

Institute for Systems Biology, 1441 N. 34th Street, Seattle, WA 98103, USA

OPEN ACCESS Freely available online

## The Complex Exogenous RNA Spectra in Human Plasma: An Interface with Human Gut Biota?

**Kai Wang**[1]*, **Hong Li**[1], **Yue Yuan**[1], **Alton Etheridge**[1,3], **Yong Zhou**[1], **David Huang**[1,3], **Paul Wilmes**[2]*, **David Galas**[1,2,3]*

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frontiers in GENETICS

**REVIEW ARTICLE**
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## The complexity, function, and applications of RNA in circulation

***Alton Etheridge***[1]***, Clarissa P. C. Gomes***[2,3]***, Rinaldo W. Pereira***[3]***, David Galas***[1,4]*** and Kai Wang***[2]*

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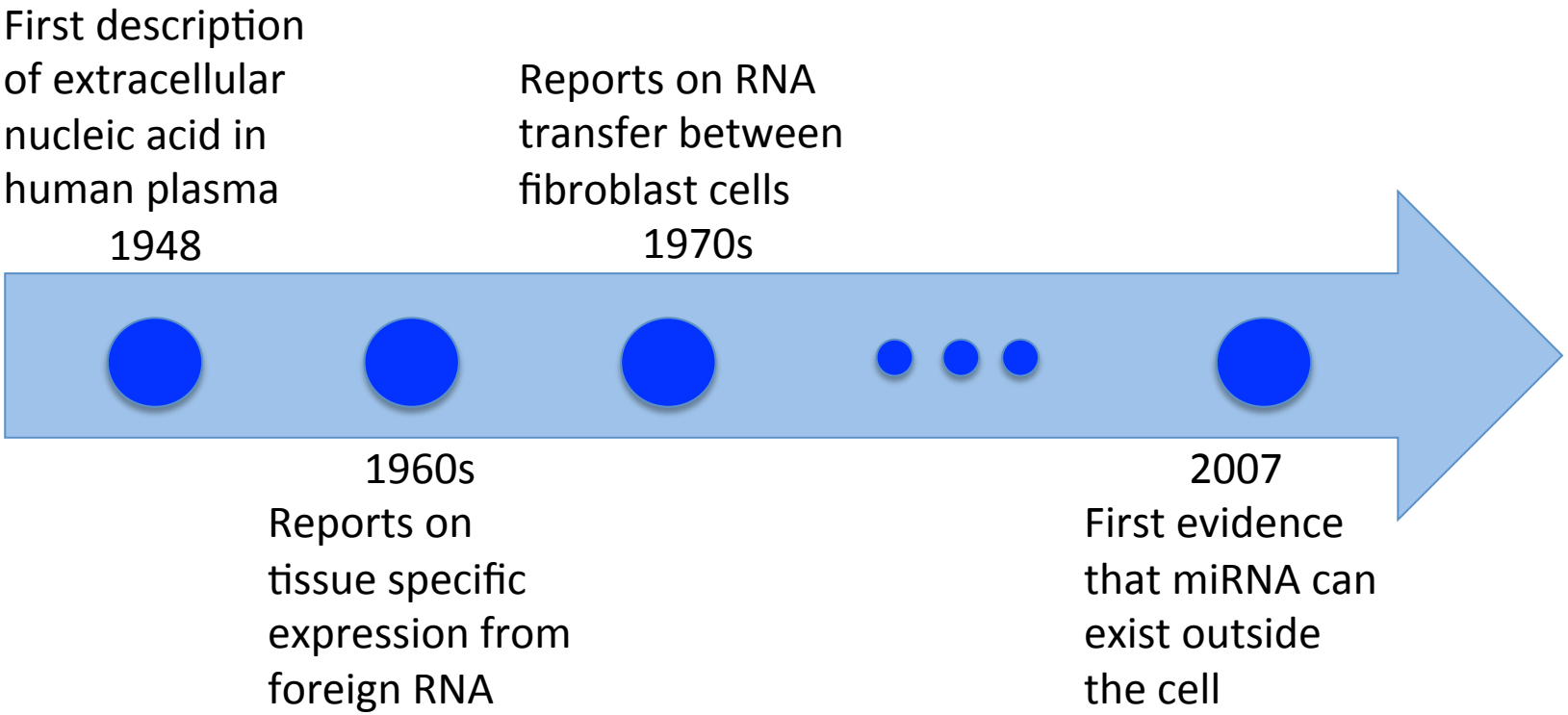
*Published online 24 May 2011* *Nucleic Acids Research, 2011, Vol. 39,*
*do*

## Characterization of extracellular circulating microRNA

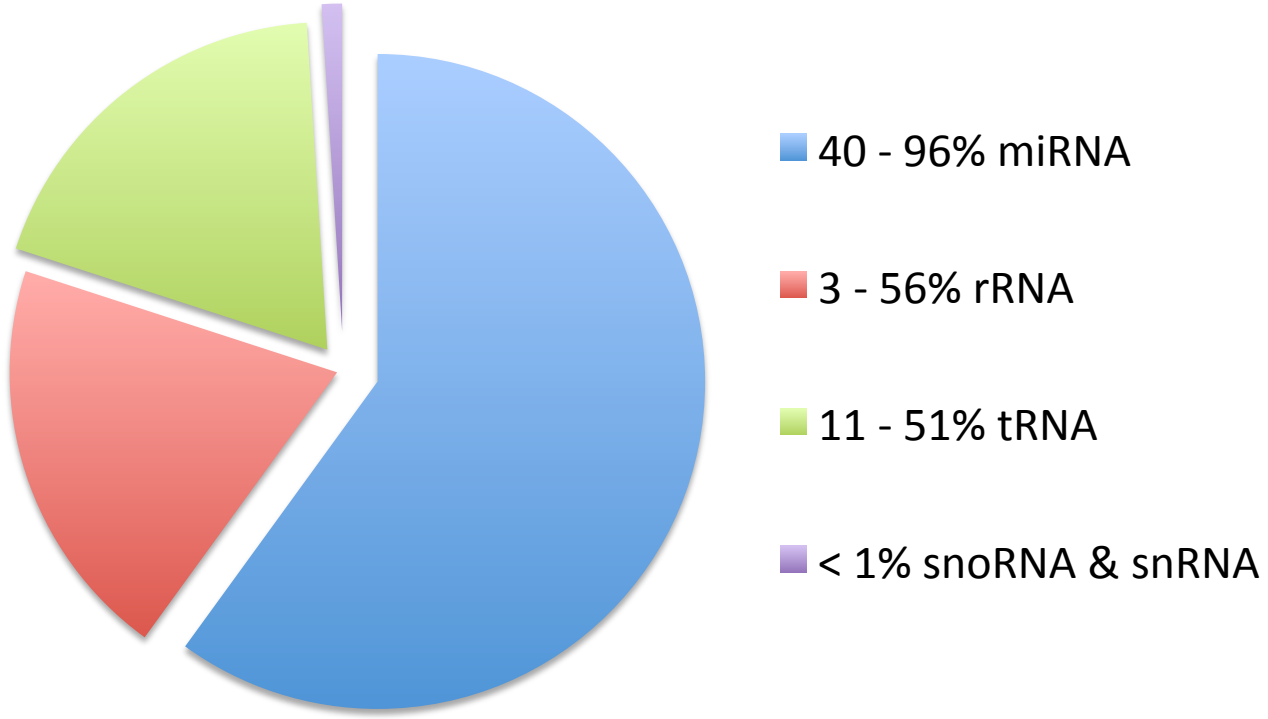
**Andrey Turchinovich**[1,2,*], **Ludmila Weiz**[3], **Anne Langheinz**[2] **and Barbara Burwinkel**[1,2]

[1]Molecular Epidemiology Group, German Cancer Research Center, Heidelberg, Germany, [2]Division of Molecular Biology of Breast Cancer, University Women's Clinic, Heidelberg, Germany and [3]Clinical Cooperation Unit Molecular Hematology/Oncology, German Cancer Research Center, Heidelberg, Germany

# Timeline


First description of extracellular nucleic acid in human plasma
1948
Reports on RNA transfer between fibroblast cells
1970s
1960s
Reports on tissue specific expression from foreign RNA
2007
First evidence that miRNA can exist outside the cell

# Extracellular RNA composition

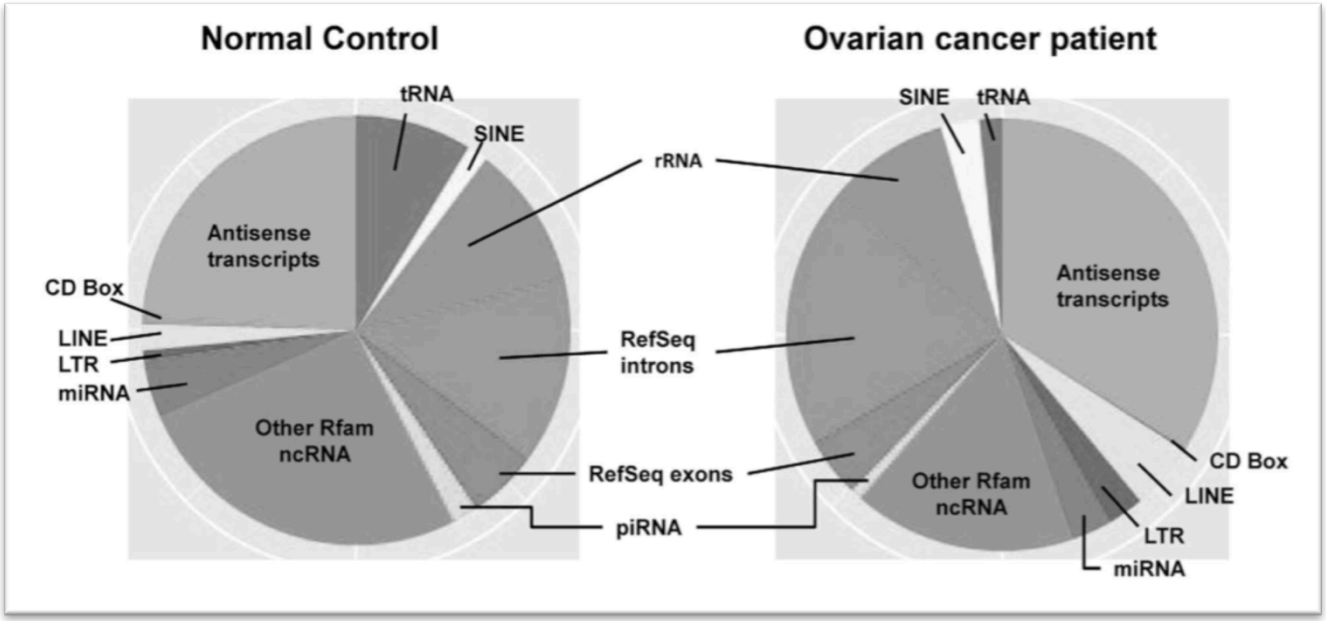


# Importance

miRNA are usually associated with diseases

(1921 mature miRNA annotated in miRBase)

**Non-invasive biomarkers.**



# Diagnostic potential

**Table 1 | Circulating miRNAs with biomarker potential to diagnose acute myocardial infarction (AMI).**

| Candidate miRNA(s) | Organism | Source | Sample size | Correlation | Reference |
|---|---|---|---|---|---|
| 1, 133a, 133b, 499-5p | Human; mouse | Plasma | 33 patients, 17 healthy; 4–5 AMI and control mice | Elevated levels distinguished cardiac damage patients from healthy subjects. Positive correlation with TnI[a] | D'Alessandra et al. (2010) |
| 1 | Human; rat | Serum | 31 patients, 20 healthy; 8 AMI rats, 8 controls | High expression was associated with CK-MB[b]; positive correlation with myocardial infarct size | Cheng et al. (2010) |
| 1 | Human | Plasma | 93 patients, 66 healthy | Up-regulation correlated with QRS[c] duration | Ai et al. (2010) |
| 208a | Human | Plasma | 33 patients, 30 healthy | Present only in patients and showed to be more specific and sensitive than TnI[a] | Wang et al. (2010a) |
| 208b, 499 | Human | Plasma | 32 patients, 36 healthy | High levels correlated with TnT[d] and CPK[e] | Corsten et al. (2010) |
| 499 | Human | Plasma | 9 patients, 10 healthy | Elevated expression correlated positively with CK-MB[b] activity | Adachi et al. (2010) |
| 1, 133a | Human | Serum | 29 patients, 42 non-AMI | Increased levels showed correlation with TnT[d] | Kuwabara et al. (2011) |
| 133, 328 | Human | Plasma, whole blood | 51 patients, 28 healthy | High levels correlated with TnT[d] | Wang et al. (2011) |
| 30c, 145, 1291, 663b | Human | Whole blood | 20 patients, 20 non-AMI | Elevated levels of 30c and 145 correlated with TnT[d]; 1291 and 663b distinguished patients from healthy subjects | Meder et al. (2011) |
| 208b, 499 | Human | Plasma | 510 patients, 87 healthy | Increased levels correlated with peak concentrations of CPK[e] and TnT[d] | Devaux et al. (2012) |

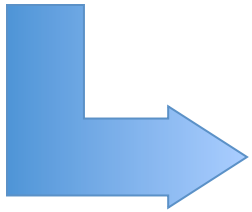
*[a]Cardiac troponin I; [b]creatine kinase muscle b; [c]QRS complex in an electrocardiogram; [d]cardiac troponin T; [e]creatine phosphokinase.*

# Extracellular environment

- miRNA was found in various body fluids: serum, plasma, saliva, tears, urine, amniotic fluid, breast milk, colostrum, bronchial lavage, cerebrospinal fluid, peritoneal fluid, pleural fluid & seminal fluid

- Synthetic RNA is degraded in **< 5s** in human plasma, but ex-mRNA in serum and plasma is stable for h**ours**

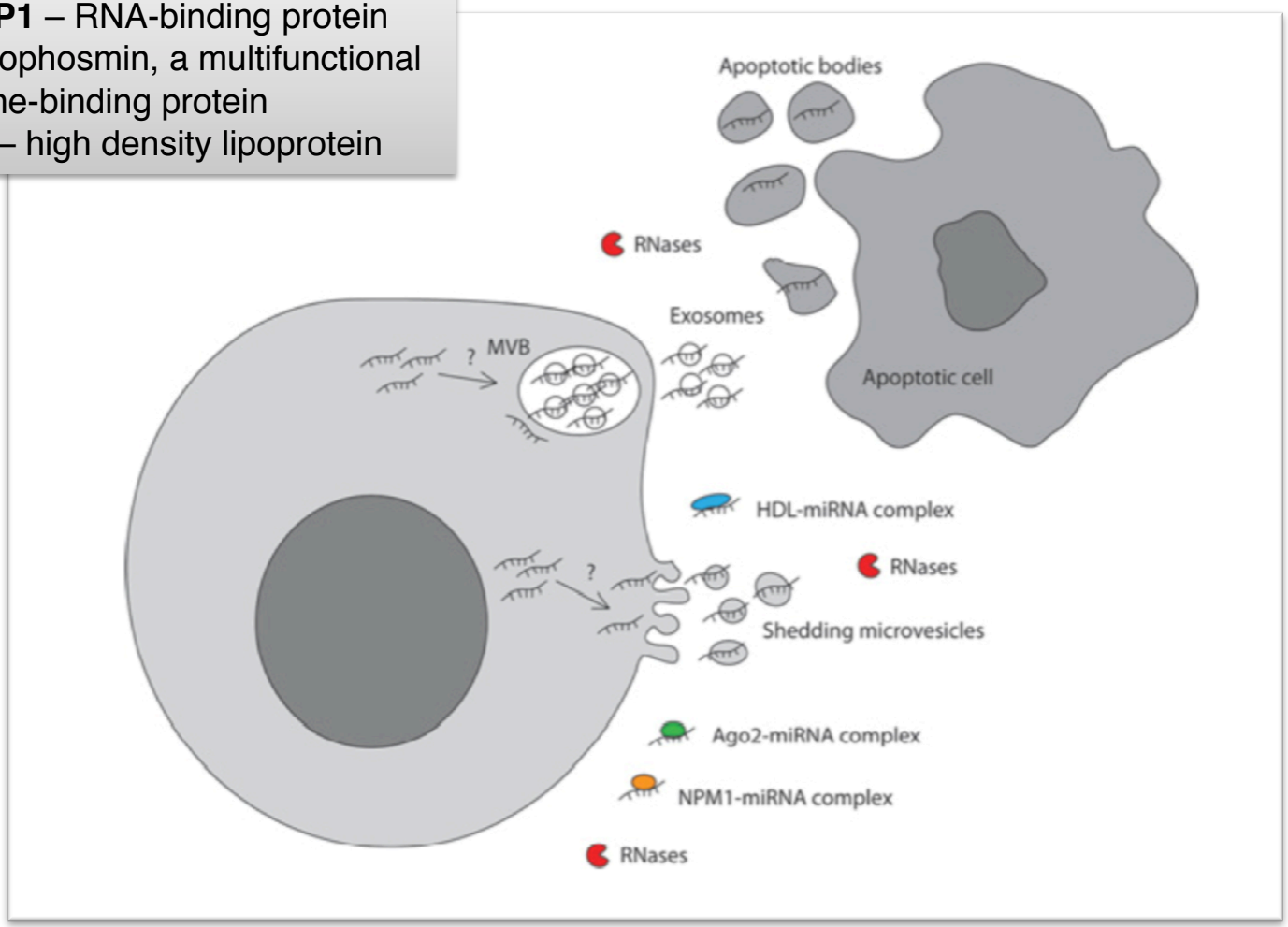
- pre-treatment of serum or plasma with detergents makes mRNAs susceptible to degradation by RNases



Exosomes

# Origins

**Ago2** – Argonaute 2
**NPMP1** – RNA-binding protein nucleophosmin, a multifunctional histone-binding protein
**HDL** – high density lipoprotein



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# Export of microRNAs and microRNA-protective protein by mammalian cells

**Kai Wang, Shile Zhang, Jessica Weber, David Baxter and David J. Galas*,†**

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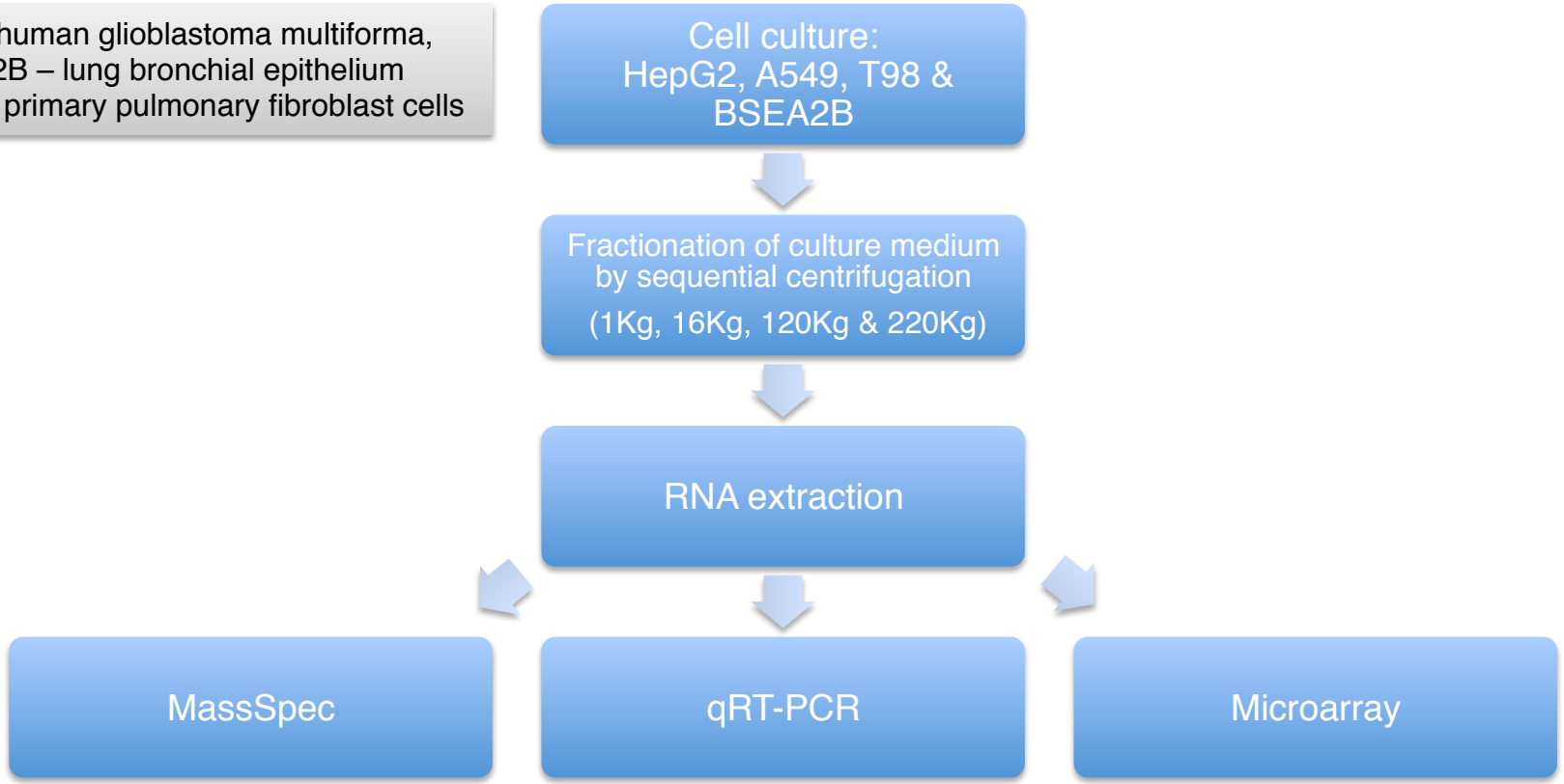
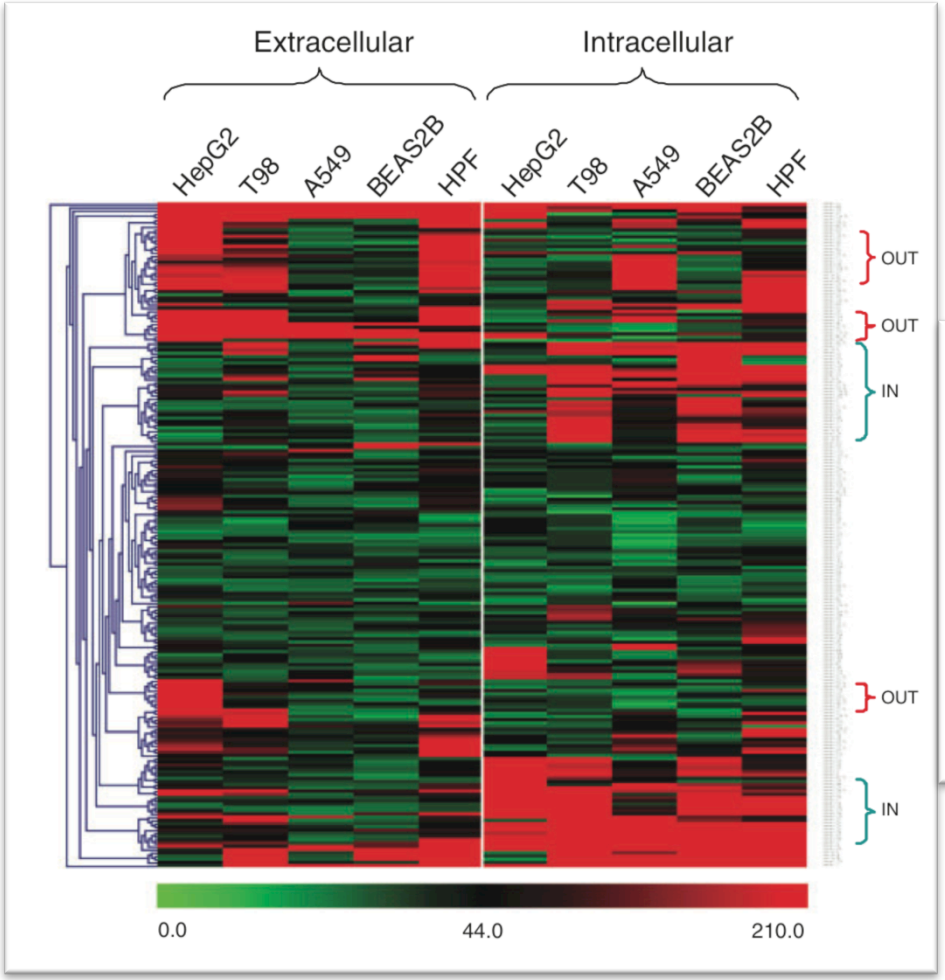
# Methods

T98 – human glioblastoma multiforma,
BEAS2B – lung bronchial epithelium
HPF – primary pulmonary fibroblast cells

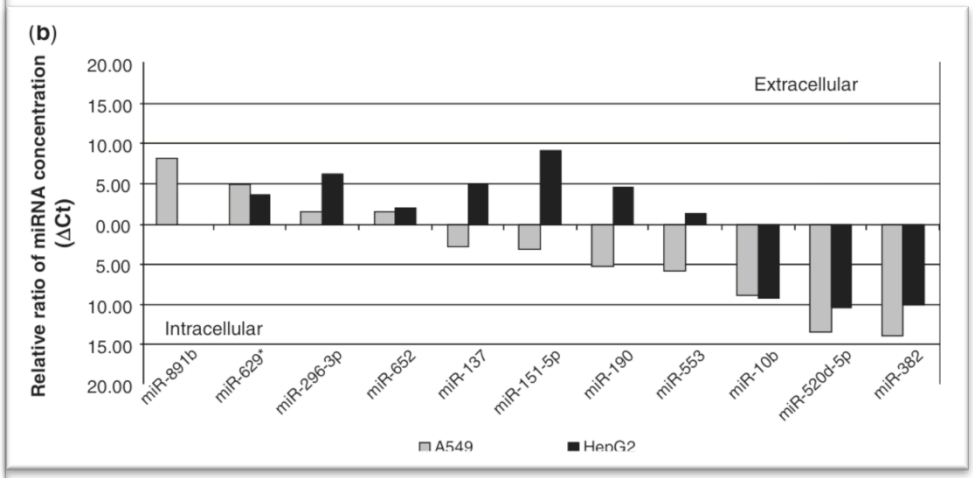


# miRNA spectra




No evidence of significant cell lysis observed for up to 48h =>

ex-miRNAs are most likely exported from intact cells by a specific cellular mechanisms



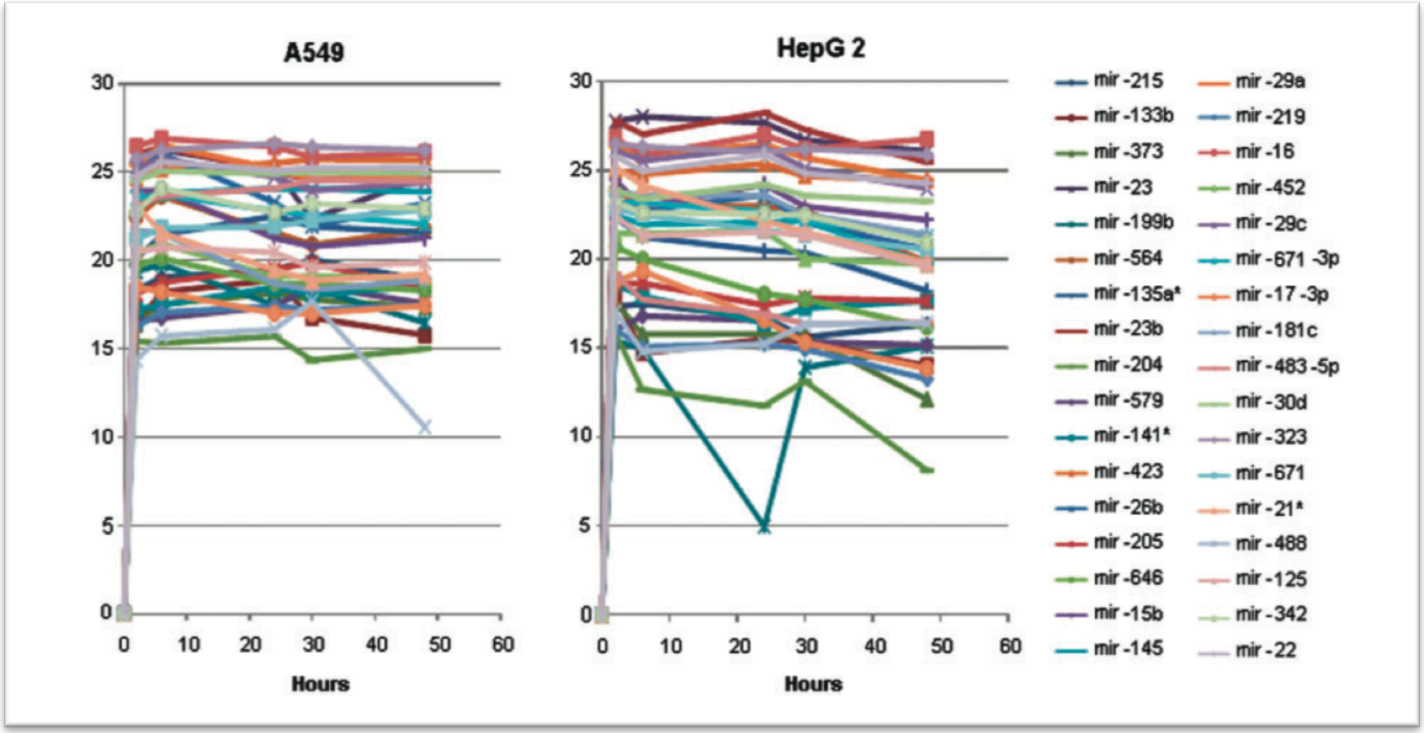
# Induction of miRNA export

- When under stress cells alter their internal states significantly leading to cell cycle arrest, and in some cases cell death.

  **Hypothesis:** cells could respond to this change of state, in part, by exporting miRNAs

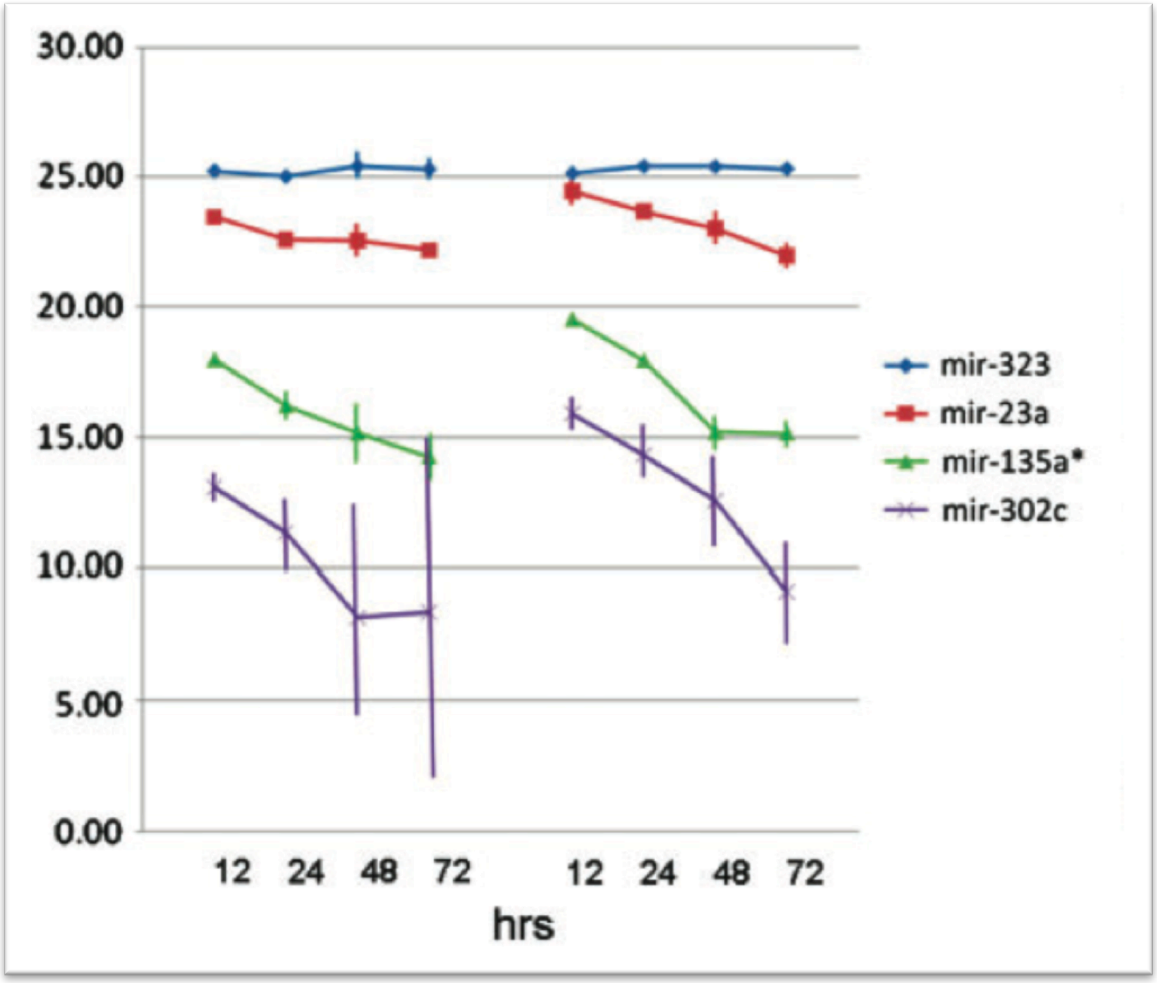
- Stress: serum deprivation

# Stress analysis results



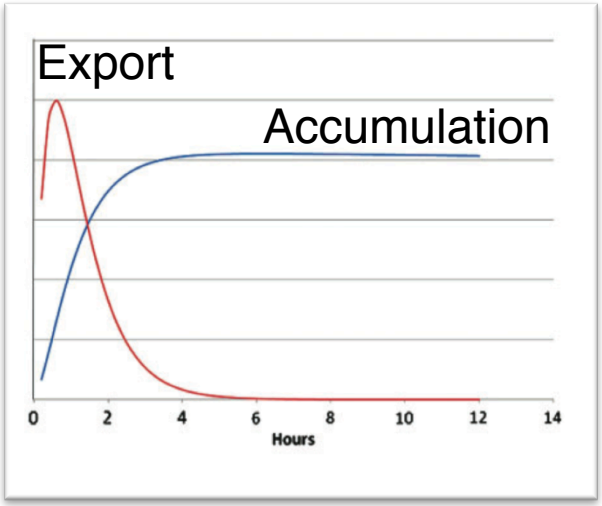

- Differences before and after serum deprivation
- Differences between cell lines

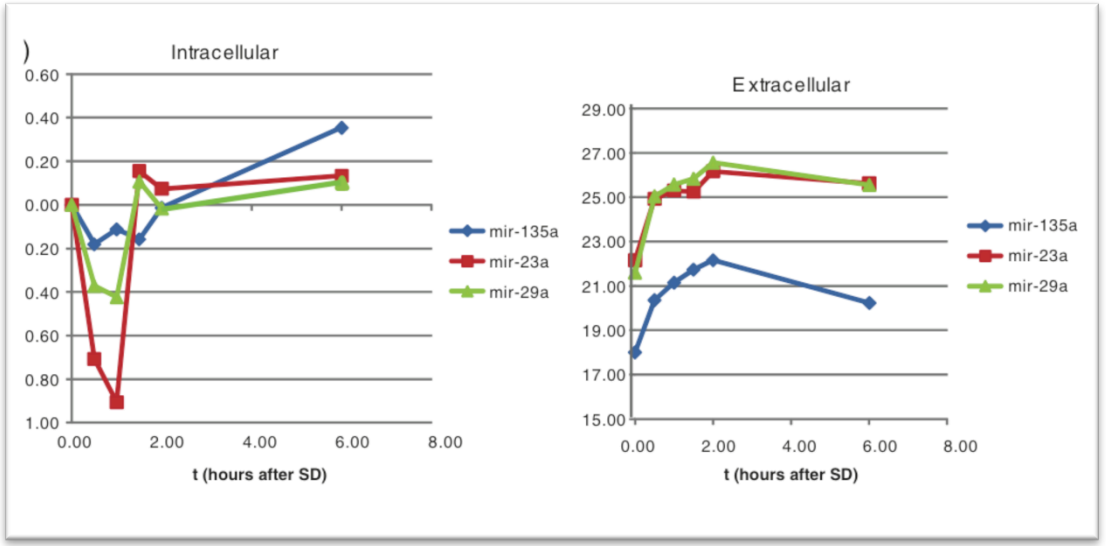
# Is exRNA stable?



# exRNA export kinetics

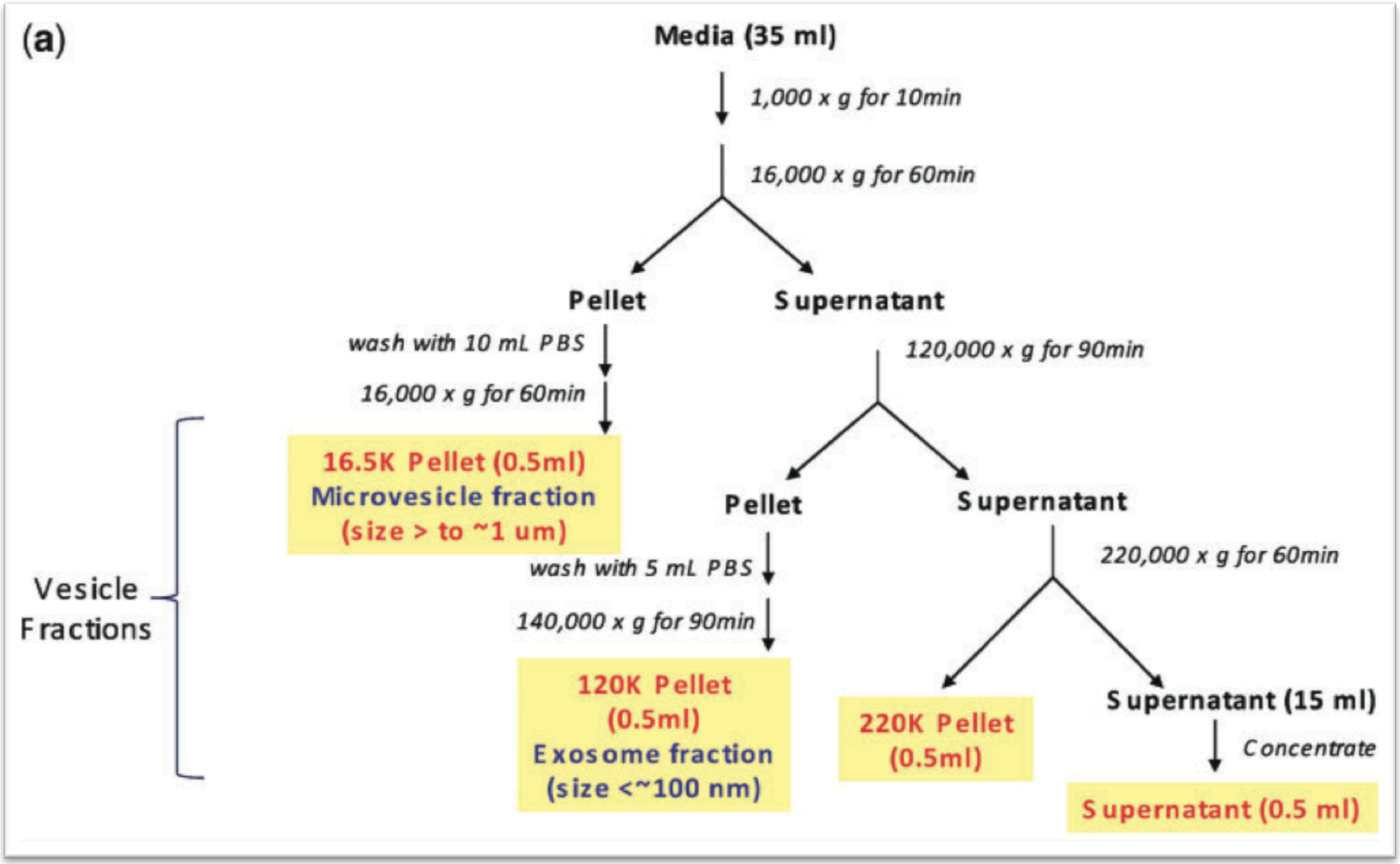
- Is the exportation of miRNA immediately after serum deprivation from a pre-synthesized miRNA pool? …



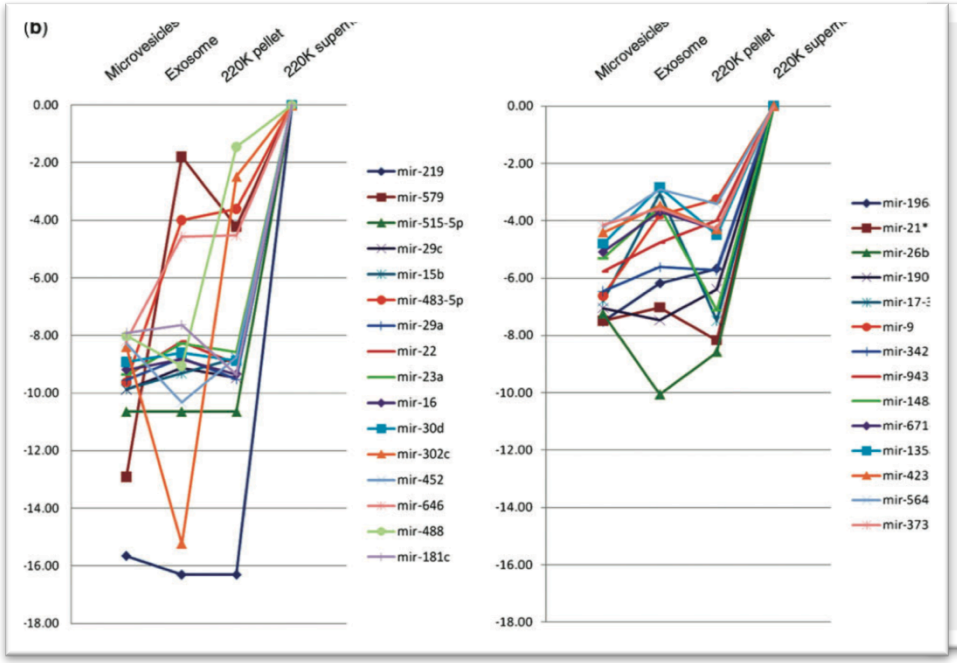



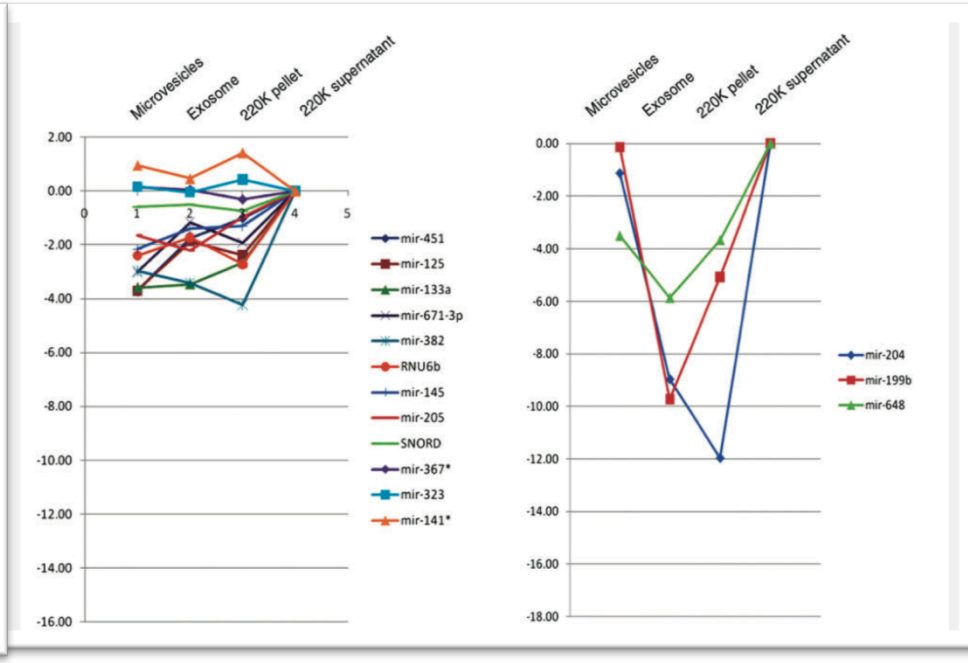

…YES

# exRNA content analysis: 1 - separation



# exRNA content analysis: 2 – results (A549)




Observations:

1. Various patterns for various miRNAs
2. miRNA content is cell specific for both extra and intracellular RNA
3. There are a couple of miRNA that are uniformly distributed regardless the fraction and cell line of provenience

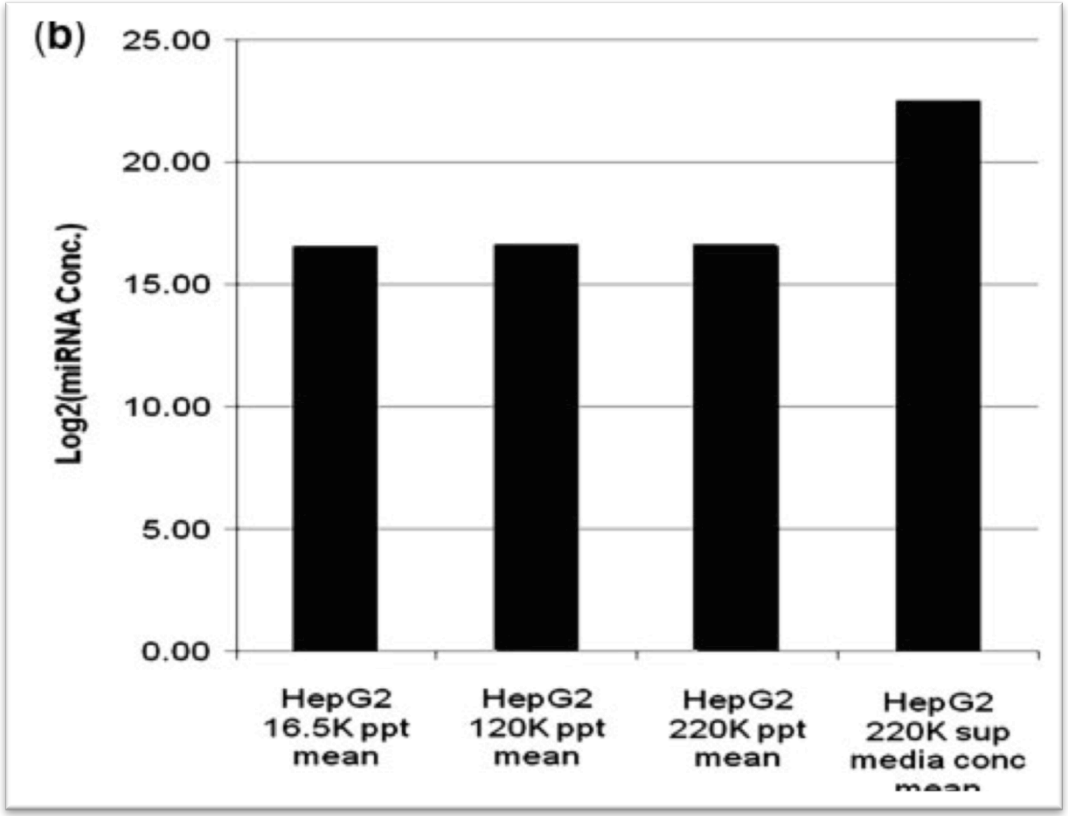
# RNA binding proteins released

**Table 1.** List of all of the known RNA-binding proteins which we observed with two or more peptide fragments in the medium (2 h after SD)

| Gene symbol | Number of peptides observed | Gene name |
|---|---|---|
| HNRNPA2B1 | 2 | Heterogeneous nuclear ribonucleoprotein a2/b1 |
| HNRPAB | 3 | Heterogeneous nuclear ribonucleoprotein a/b |
| ILF2 | 2 | Interleukin enhancer binding factor 2, 45 kda |
| NCL | 7 | Nucleolin |
| NPM1 | 4 | Nucleophosmin (nucleolar phosphoprotein b23, numatrin) |
| RPL10A | 2 | Ribosomal protein l10a |
| RPL5 | 2 | Ribosomal protein l5 |
| RPLP1 | 6 | Ribosomal protein, large, p1 |
| RPS12 | 2 | Ribosomal protein s12 |
| RPS19 | 2 | Ribosomal protein s19 |
| SNRPG | 2 | Small nuclear ribonucleoprotein polypeptide g |
| TROVE2 | 2 | Trove domain family, member 2 |

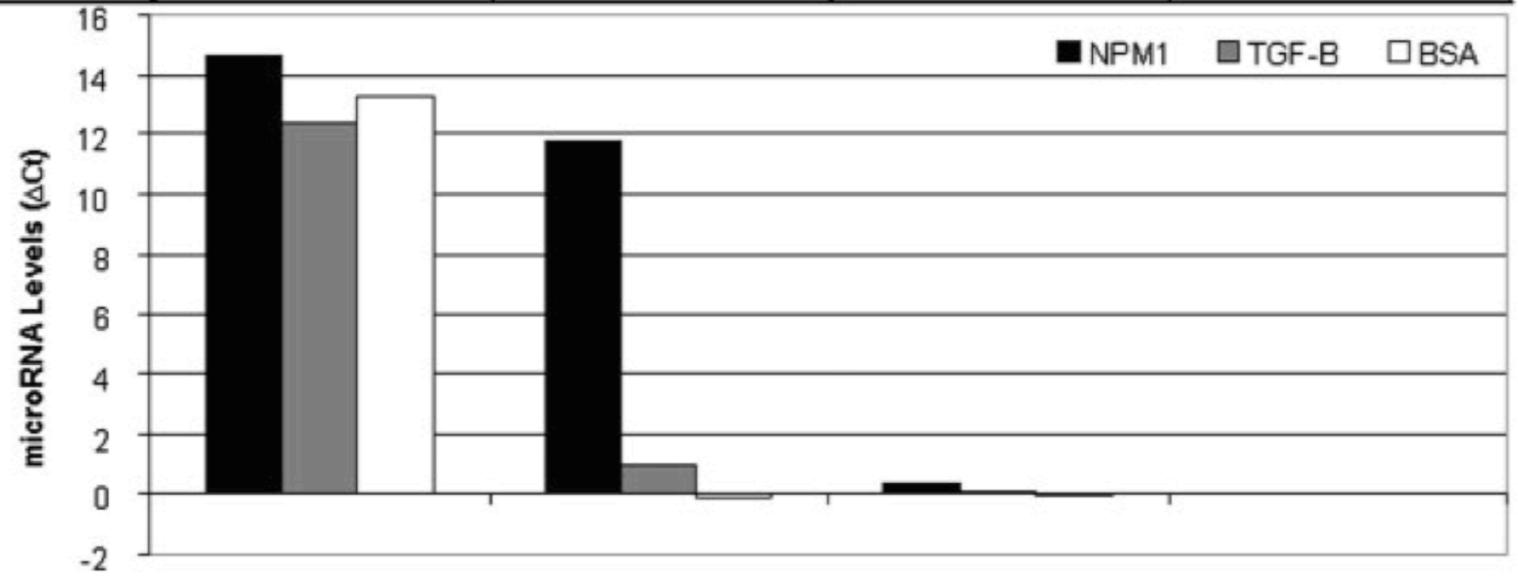
# NPM1

- NPM1 (nucleophosmin 1) is a nucleolar RNA-binding protein,
- Nucleophosmin 1 is implicated in the nuclear export of the ribosome
- Large quantities of NPM1 were observed outside of the cell
- NPM1 bound miRNA is protected from RNase degradation



# Protein Protection

| | | | | |
|---|---|---|---|---|
| miRNA | + | + | + | - |
| Protein | + | + | - | + |
| RNAse A | - | + | + | + |



# Conclusions

- The exRNA spectrum varies upon cell provenience and/or stress
- ex-miRNA are exported after stress
- exRNA export is energy dependent
- exRNA is either in vesicle or protein bound (NPM1)
- The export of exRNA is a mean of cell-cell communication

# Remarks

- Are RNA Binding Proteins promiscuous or specific for exRNA?
- Do have exRNA have specific binding motifs?
- exRNA purification & identification is limited experimentally => How does this affect the data analysis ?

# Data availability


OPEN ACCESS Freely available online

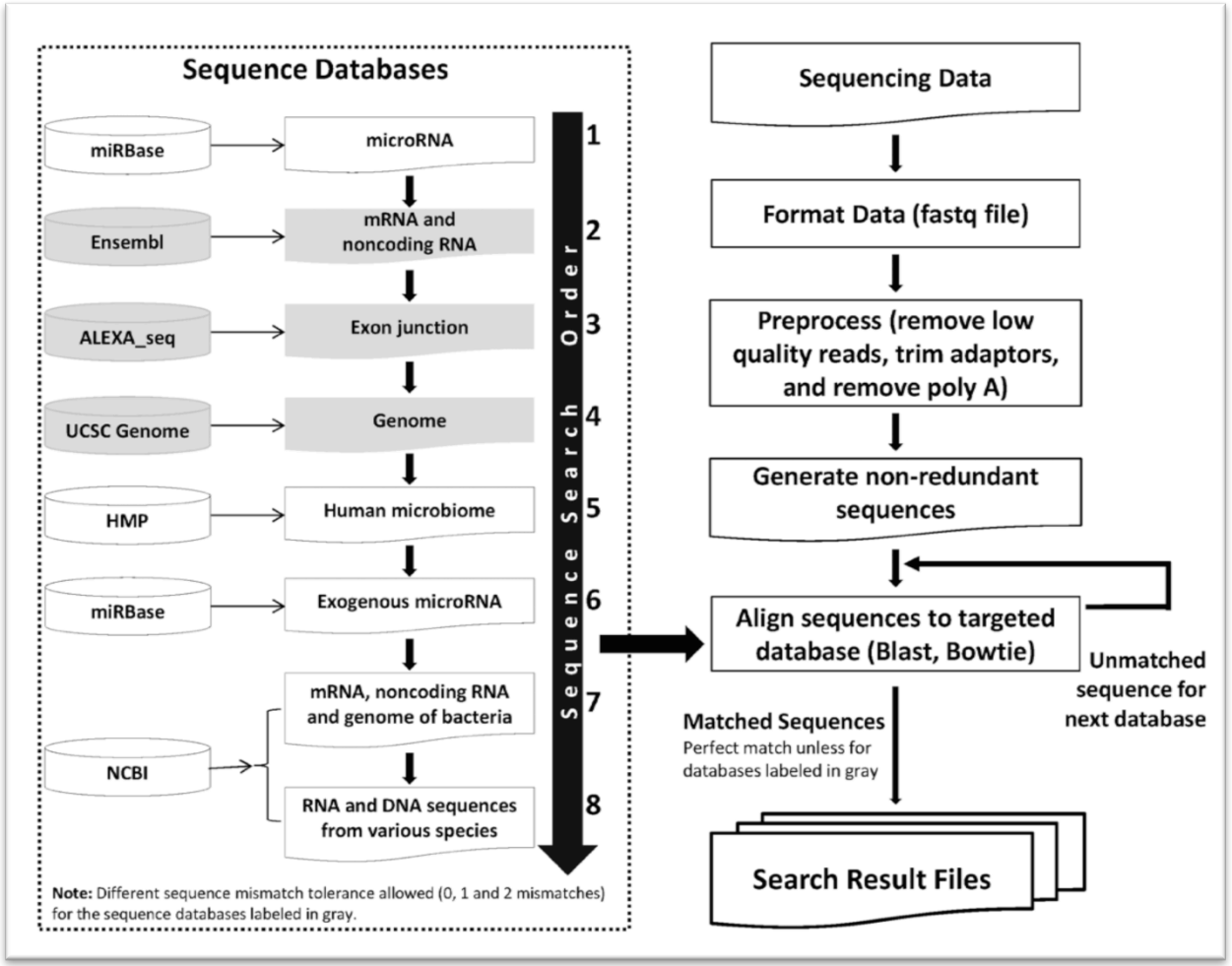
PLOS ONE

# The Complex Exogenous RNA Spectra in Human Plasma: An Interface with Human Gut Biota?

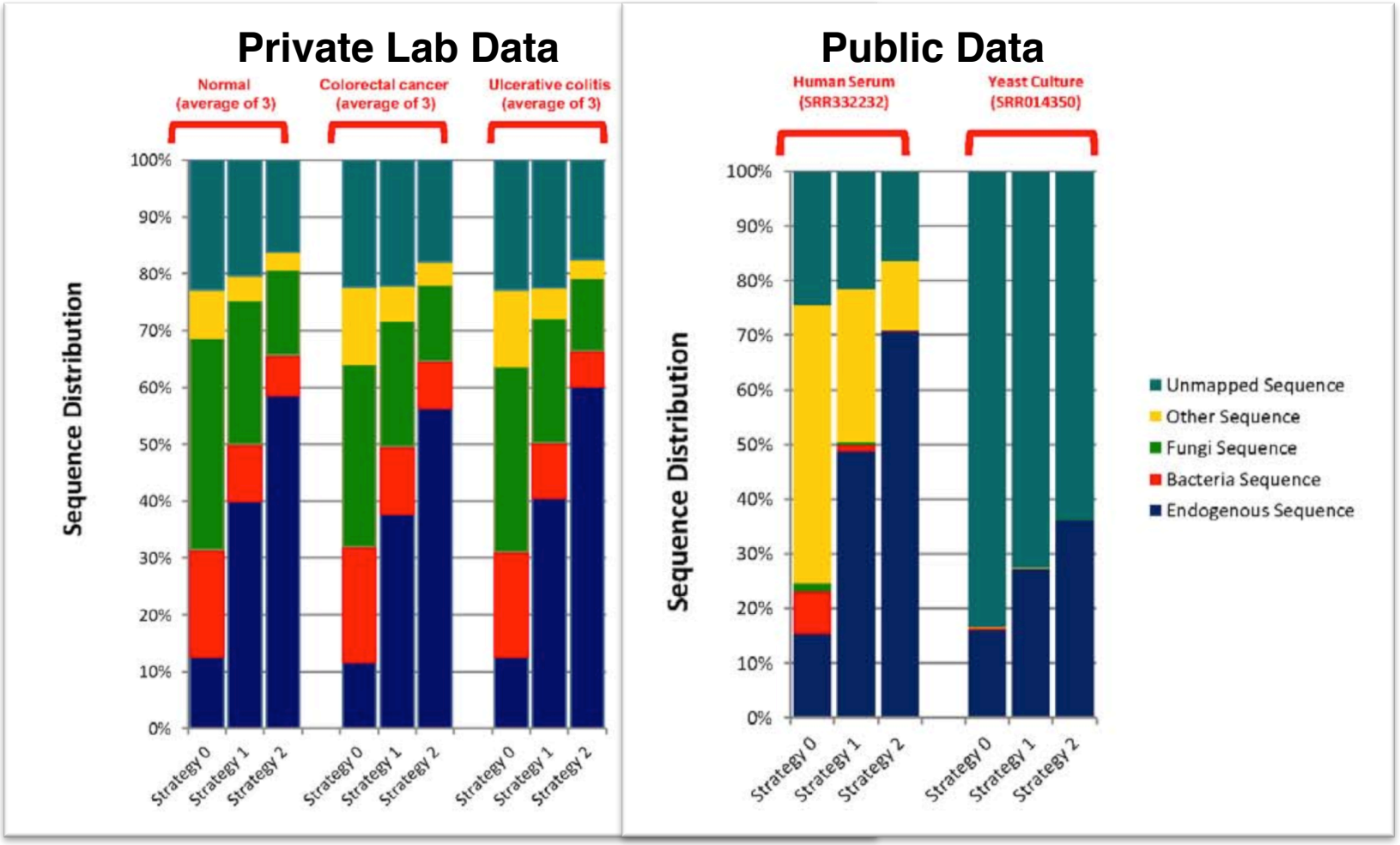
**Kai Wang[1]*, Hong Li[1], Yue Yuan[1], Alton Etheridge[1,3], Yong Zhou[1], David Huang[1,3], Paul Wilmes[2]*, David Galas[1,2,3]***

**1** Institute for Systems Biology, Seattle, Washington, United States of America, **2** Luxembourg Center for Systems Biomedicine, University of Luxembourg, Luxembourg City, Luxembourg, **3** Pacific Northwest Diabetes Research, Seattle, Washington, United States of America

# Sequence mapping



# Data quality



# Remarks

- Experimental bottleneck: separation and identification of exogenous and endogenous RNA

# Thank You!