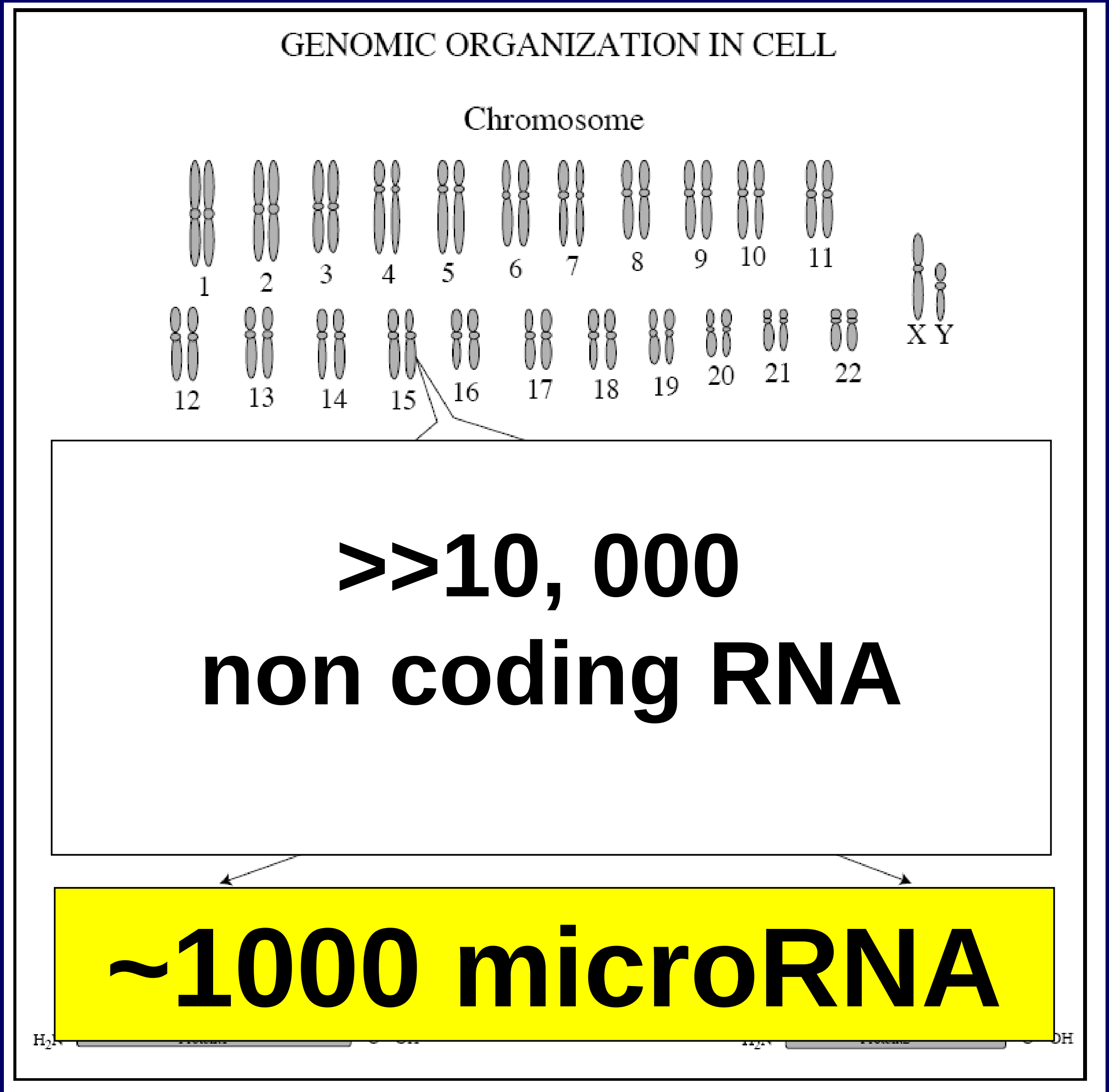
microRNAs (miRNA) and Biomarkers

“Small RNAs Make Big Splash”

- miRNAs & Genome
- Function
- Biomarkers in Cancer
- Future Prospects

Javed Khan M.D.
National Cancer Institute
EORTC-NCI-ASCO
November 2007

The Human Genome and Proteome



>>10, 000
non coding RNA

~1000 microRNA

23 pairs of Chromosomes

3,000,000,000 bp

20-25,000 genes

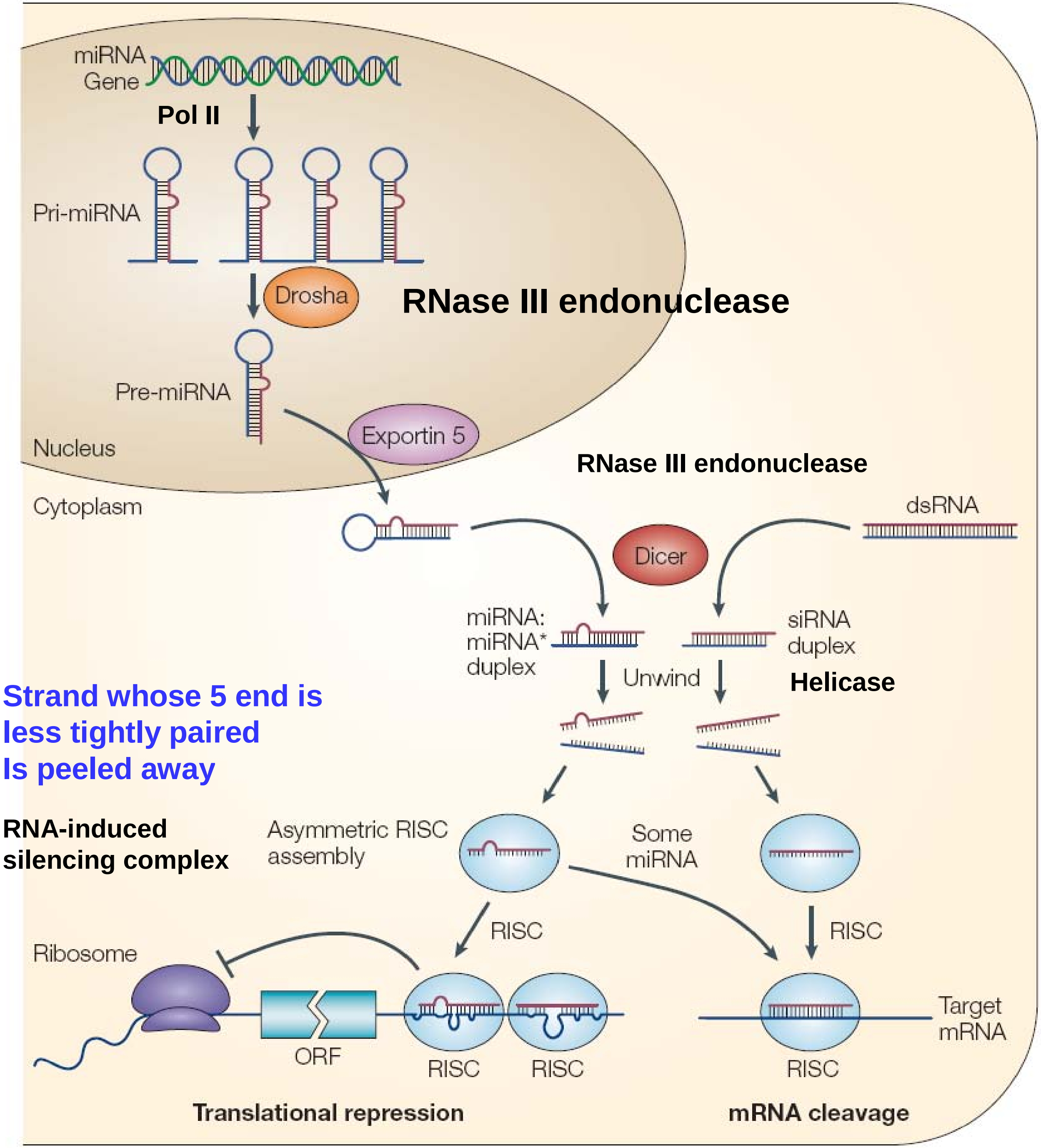
>10,000 splice variants

>100,000 Proteins

MicroRNA (miRNA)-Overview

- **Single-stranded non-coding RNAs of ~22bp nucleotides that negatively regulate gene expression**
- **Discovered in 1993 Lee..Ambros et al. *lin-4* in *C.elegans***
- **microRNA described in 2001 Tuschl, Bartel, Ambros**
- **Estimated ~1000 in human genome (722 in Sanger Database v10) (http://microrna.sanger.ac.uk/sequences/index.shtml)**
- **1-2% of expressed genes**
- **Mostly found within introns of genes**
- **Functions include regulation of cellular growth, death, metabolism, differentiation and development**
- **Potential Biomarkers and Therapeutic Targets for cancer**

Lin He and Gregory J. Hannon
doi:10.1038/nrg1379
JULY 2004 | VOLUME 5



RNase III endonuclease

RNase III endonuclease

Helicase

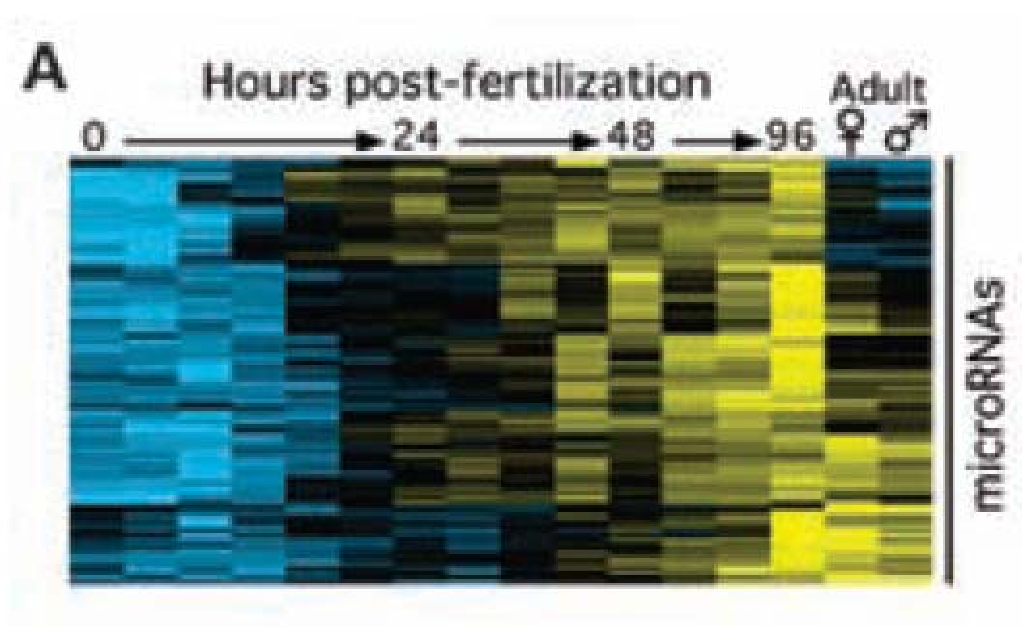
Strand whose 5 end is less tightly paired Is peeled away

RNA-induced silencing complex

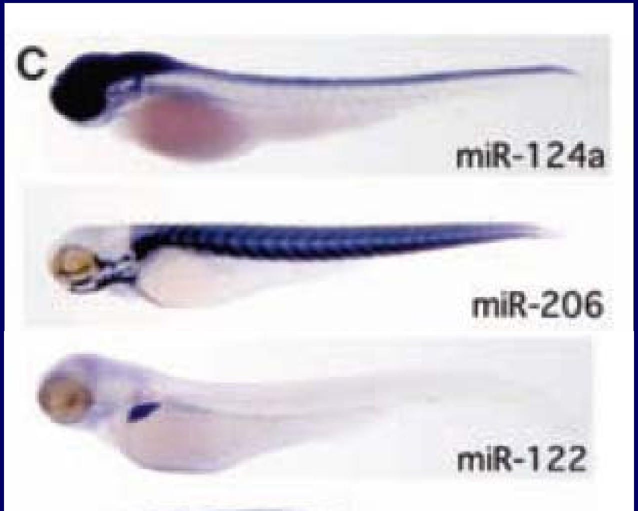
1. **miRNAs that bind with <u>perfect or nearly perfect</u> complementarity to protein coding mRNA sequences induce the RNA-mediated interference (RNAi) pathway causing <u>mRNA degradation</u>.**
2. **<u>Imperfect complimentary</u> binding to 3'-UTR results <u>in translational repression.</u>**
3. **Imperfect 3'-UTR binidng also leads to mRNA degradation.**
4. **Net effect is suppression of 200+ proteins for each miRNA**
5. **Multiple miRNAs target single mRNA**
6. **Estimated control of 1/3 of all mRNAs**

MicroRNA expression is tissue specific during segmentation and later stages but not early in development:

∴microRNAs are involved in differentiation and maintenance of tissue identity not in tissue fate



Blue=low, Black=mean, Yellow=high



Nervous System

Muscle

Liver

72 hrs post fertilization

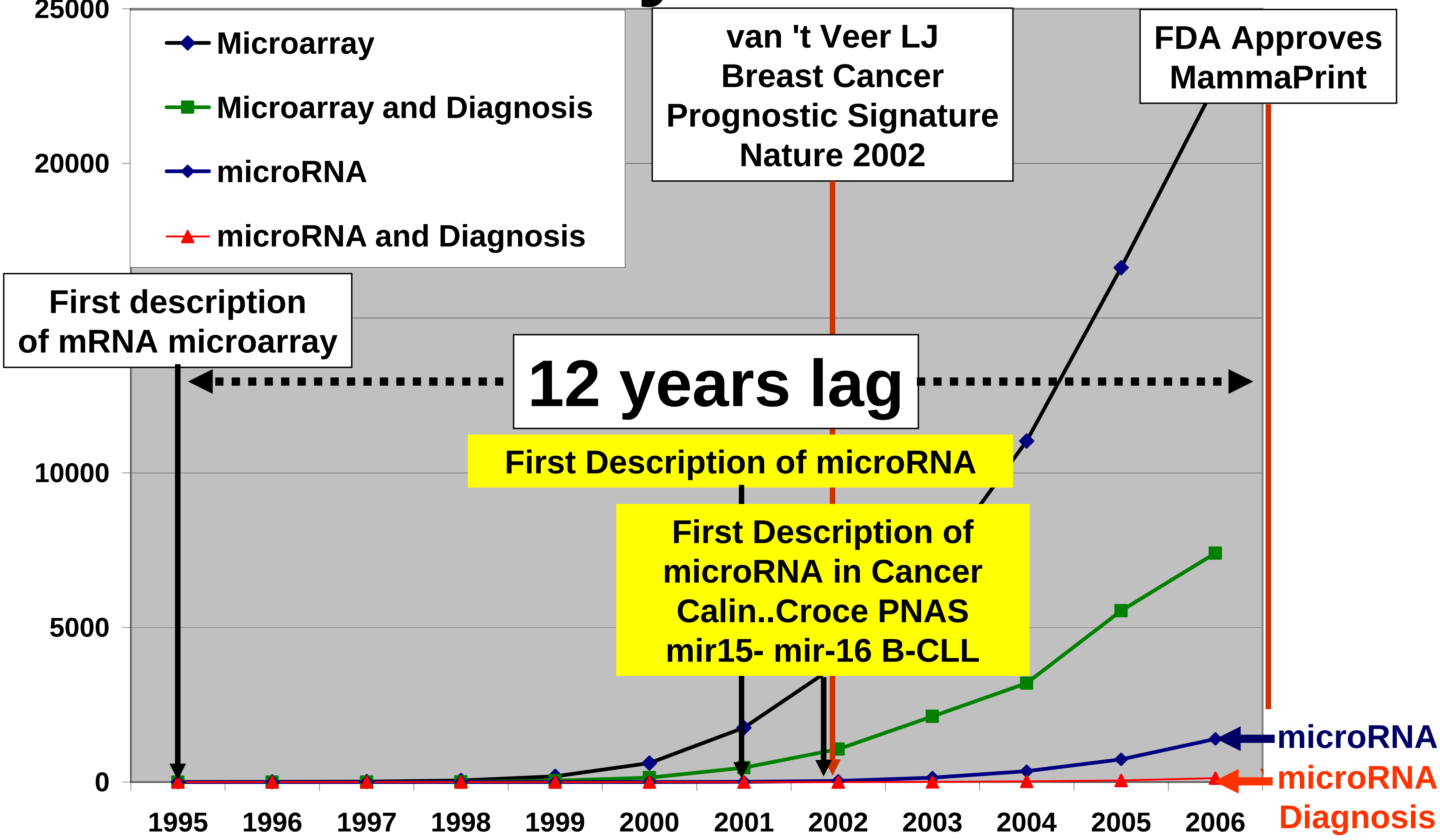
Erno Wienholds et al. 2005, Science 10.1126/science.1114519

MicroRNA Profiling and Cancer

Advantages for using microRNA as Biomarkers to Diagnose Cancer

- Relative small number (1,000 *vs.* 20-25,000)
- Maybe more informative than mRNA profiling for diagnosing cancers
- Better preserved in paraffin
- Potential mechanism for diseases
- Potential therapeutic agents or targets

Microarray Publication



van 't Veer LJ
Breast Cancer
Prognostic Signature
Nature 2002

FDA Approves
MammaPrint

First description
of mRNA microarray

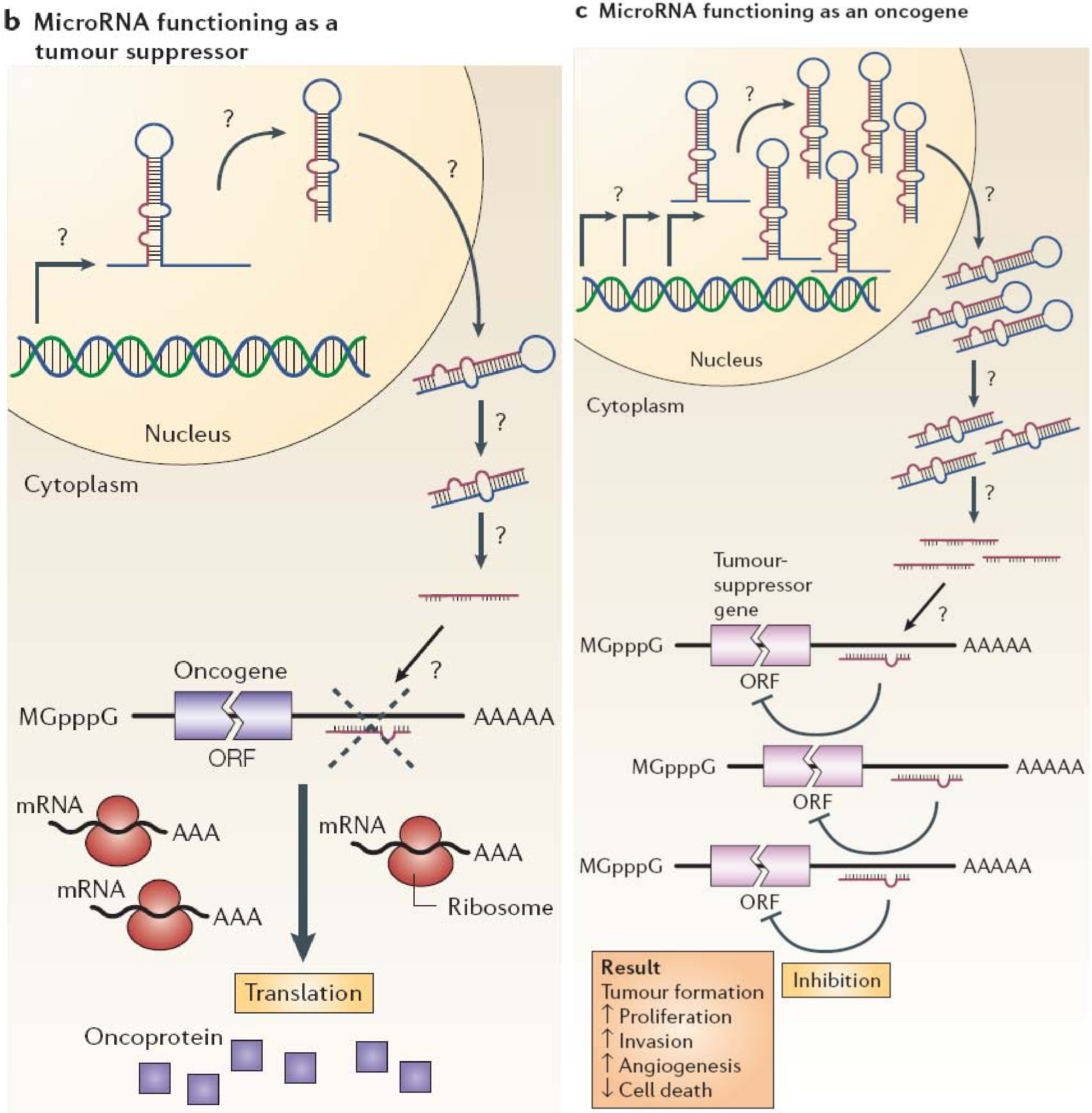
12 years lag

First Description of microRNA

First Description of
microRNA in Cancer
Calin..Croce PNAS
mir15- mir-16 B-CLL

MicroRNAs as Tumor Suppressors

MicroRNAs as Oncogenes



b MicroRNA functioning as a tumour suppressor

c MicroRNA functioning as an oncogene

Result

Tumour formation

↑ Proliferation

↑ Invasion

↑ Angiogenesis

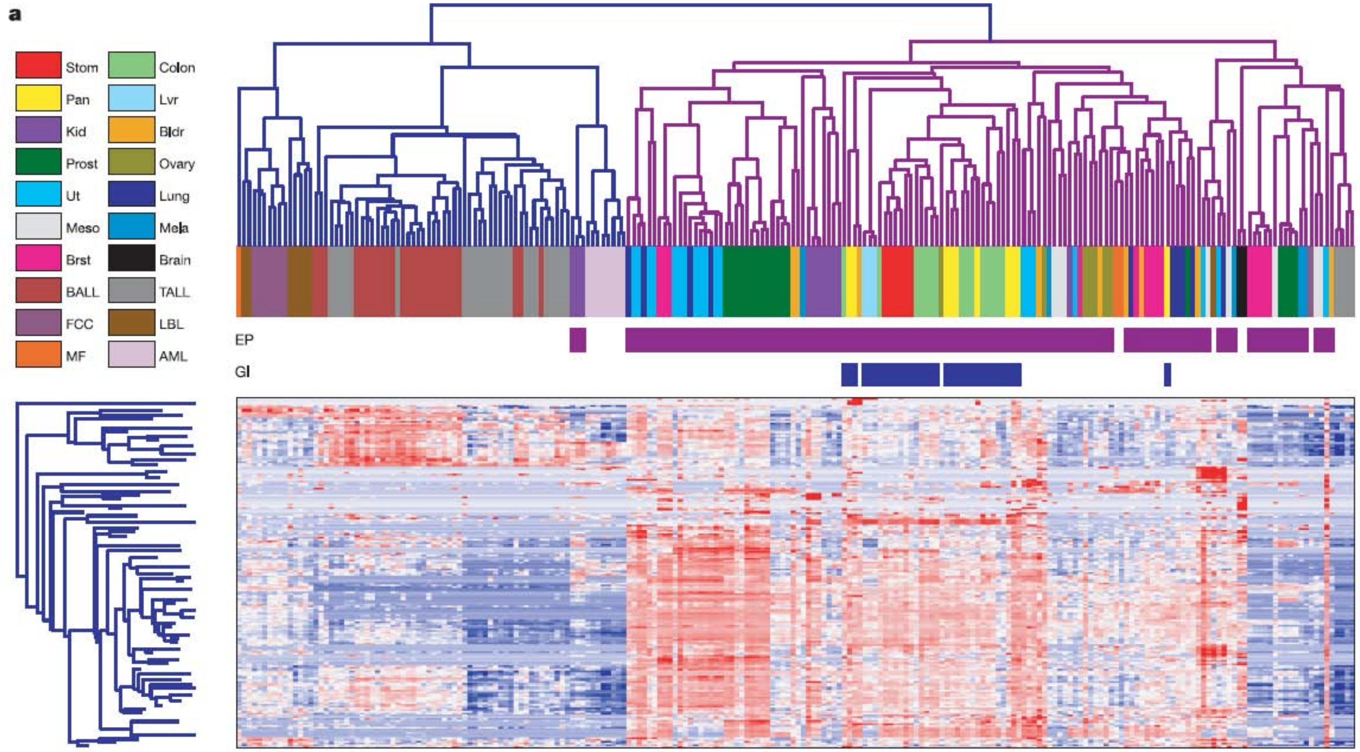
↓ Cell death

Esquela-Kerscher, et al.
Can. Nat Rev Vol. 6, Apr. 2006

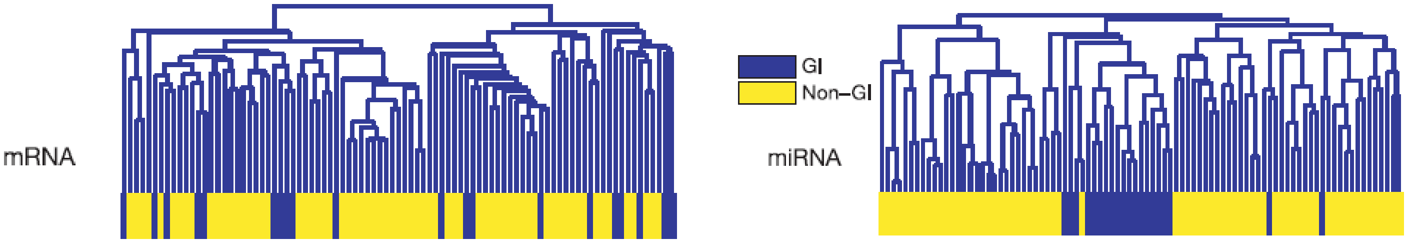
MicroRNA expression profiles classify human cancers

Jun Lu[1,4*], Gad Getz[1*], Eric A. Miska[2*†], Ezequiel Alvarez-Saavedra[2], Justin Lamb[1], David Peck[1], Alejandro Sweet-Cordero[3,4], Benjamin L. Ebert[1,4], Raymond H. Mak[1,4], Adolfo A. Ferrando[4], James R. Downing[5], Tyler Jacks[2,3], H. Robert Horvitz[2] & Todd R. Golub[1,4,6]

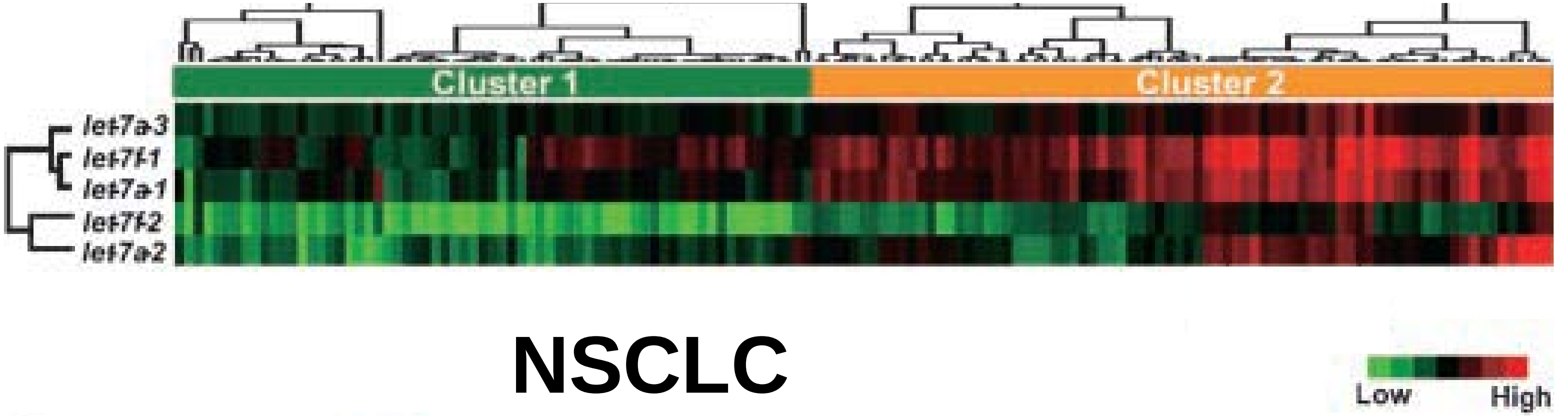
Vol 435|9 June 2005|**doi:10.1038/nature03702**



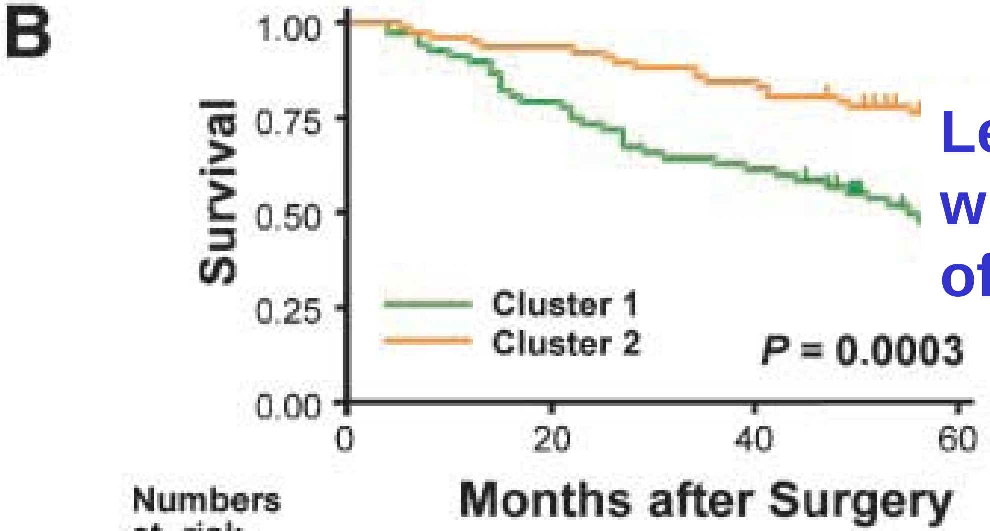
1. **miRNA profile reflected developmental lineage and differentiation.**
2. **Down regulation in tumor cf. normal tissue**
3. **miRNA correctly classified 12 of 17 cancers of unknown primaries, whereas the mRNA analysis only correctly classified one sample**



High expression of microRNAs Let-7 family associated with good prognosis



NSCLC



Numbers at risk				
Cluster 1	67	53	41	21
Cluster 2	76	71	63	45

Let-7 targets or suppresses RAS which is mutated in 15-39% of human cancers

Takamizawa et al
CANCER RESEARCH 64, 3753–3756, June 1, 2004

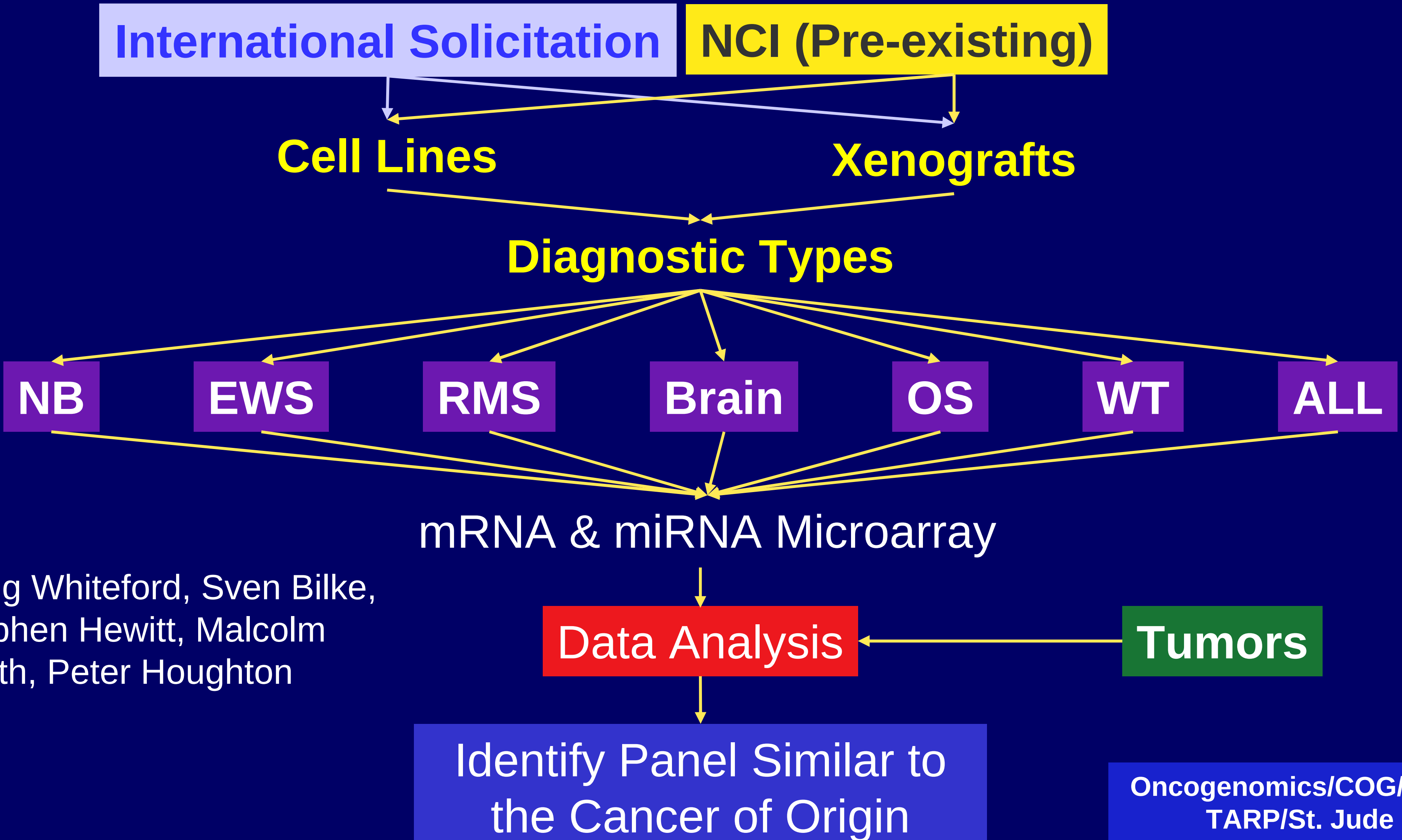
microRNA Profiling of Pediatric Malignancies

Systematic Approach to Identify Pre-Clinical Models for Drug Screening for Pediatric Cancers using Genomics

Main Objectives

1. To identify a panel of pediatric cancer models (xenograft and cell lines) that most closely resembles the cancer of origin
2. To identify diagnostic biomarkers for pediatric malignancies
3. To identify novel therapeutic molecular targets for children with cancer
4. Use these validated pre-clinical models to screen drugs to prioritize which drugs to take to the clinic

Systematic Approach to Identify Pre-Clinical Models for Drug Screening for Pediatric Cancers using Genomics



International Solicitation

NCI (Pre-existing)

Cell Lines

Xenografts

Diagnostic Types

NB

EWS

RMS

Brain

OS

WT

ALL

mRNA & miRNA Microarray

Data Analysis

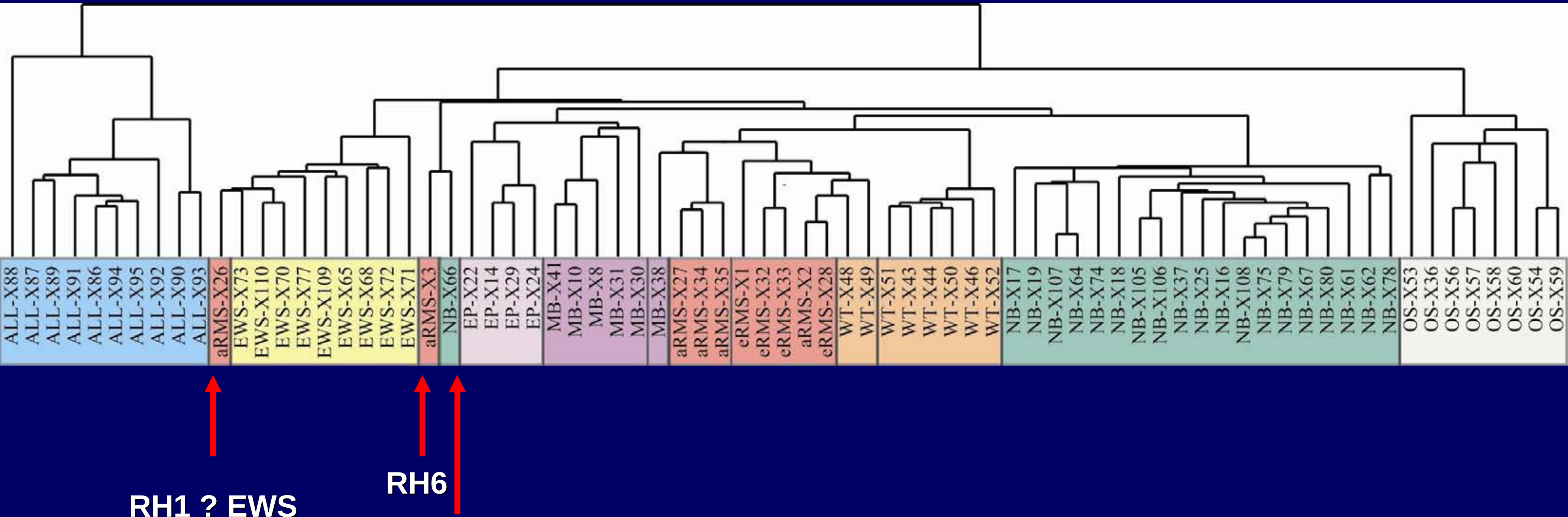
Tumors

Identify Panel Similar to the Cancer of Origin

Craig Whiteford, Sven Bilke, Stephen Hewitt, Malcolm Smith, Peter Houghton

Oncogenomics/COG/CTEP TARP/St. Jude

Xenografts Cluster Along Diagnostic Type by Unsupervised Clustering using cDNA microarrays to profile messenger RNA with all 38,789 Good Quality Probes



RH6

RH1 ? EWS

JHAN

Cancer Res. 2007; 67(1):32-40

miRNA Profiling-Goals

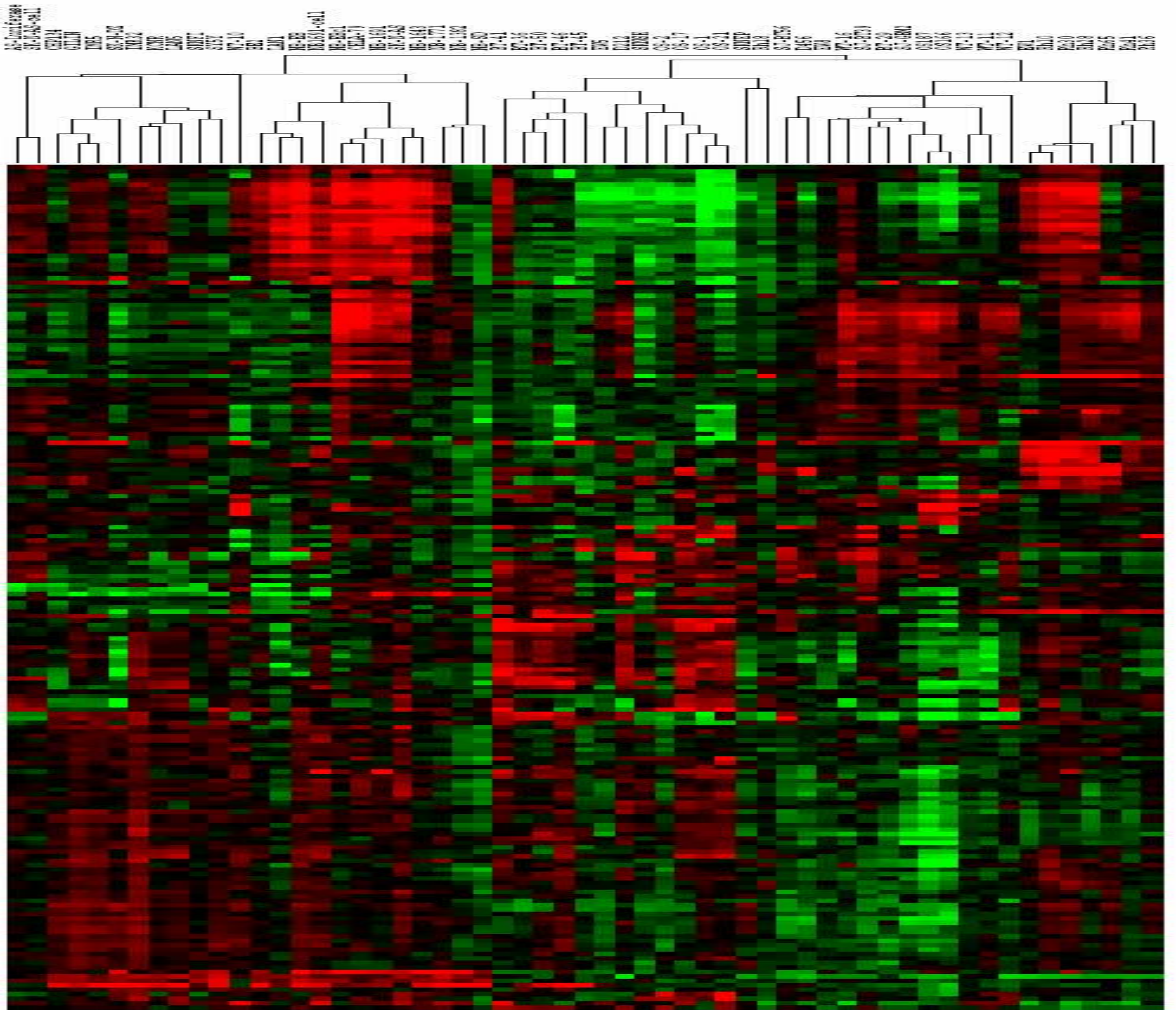
- Identify tumor-type-specific miRNAs
    - Tumor Classification
    - Diagnosis

- Identify miRNA targets
    - Biology
    - Potential therapy

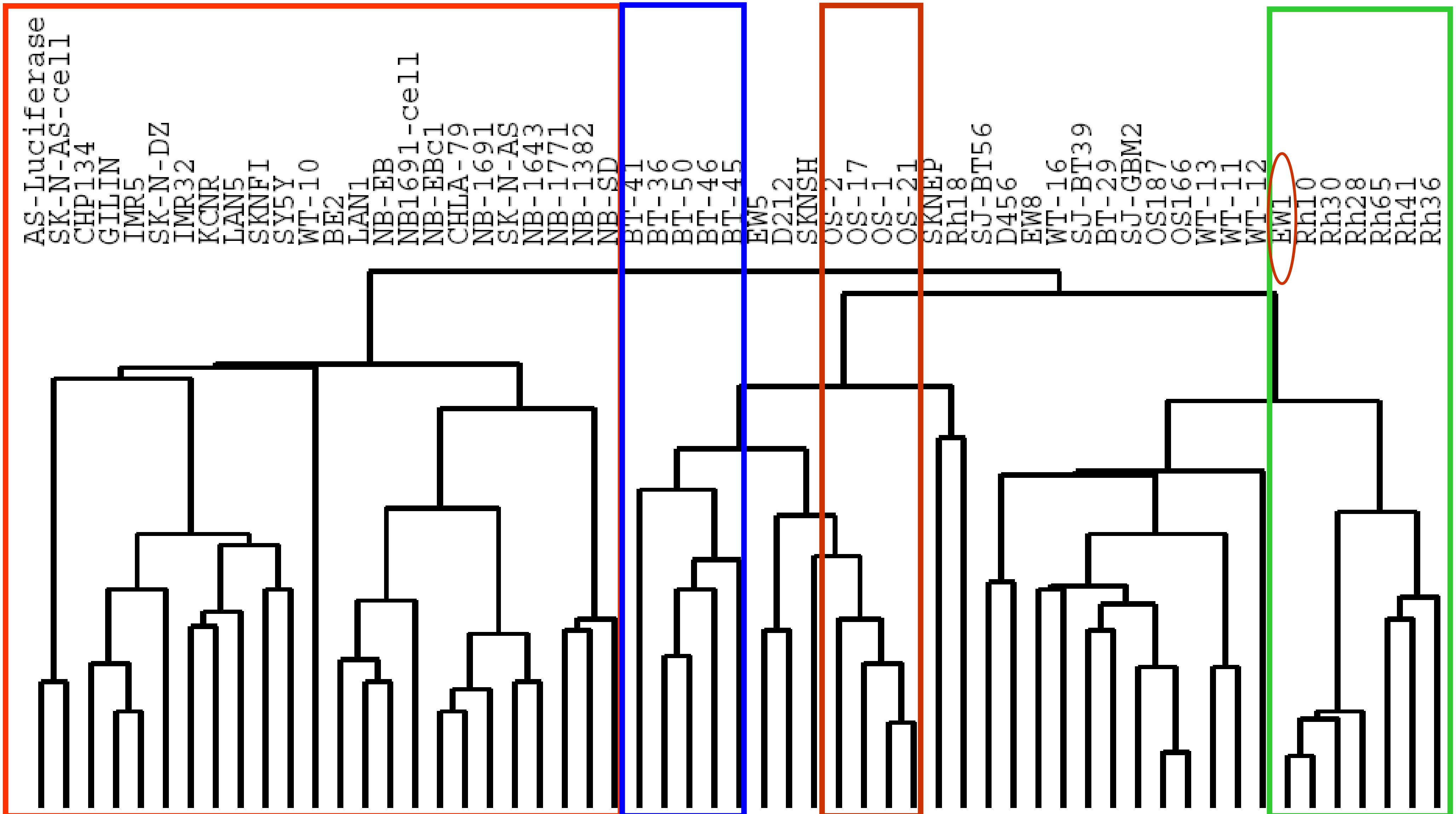
MicroRNA Array Design

- Probe design: all human miRNAs from Sanger miRNA Registry
- Total 648 miRNAs (Sanger 722 V10)
- 18 control probes for small RNA (tRNA, U4, U6, U21, etc, PGK1, b-actin)

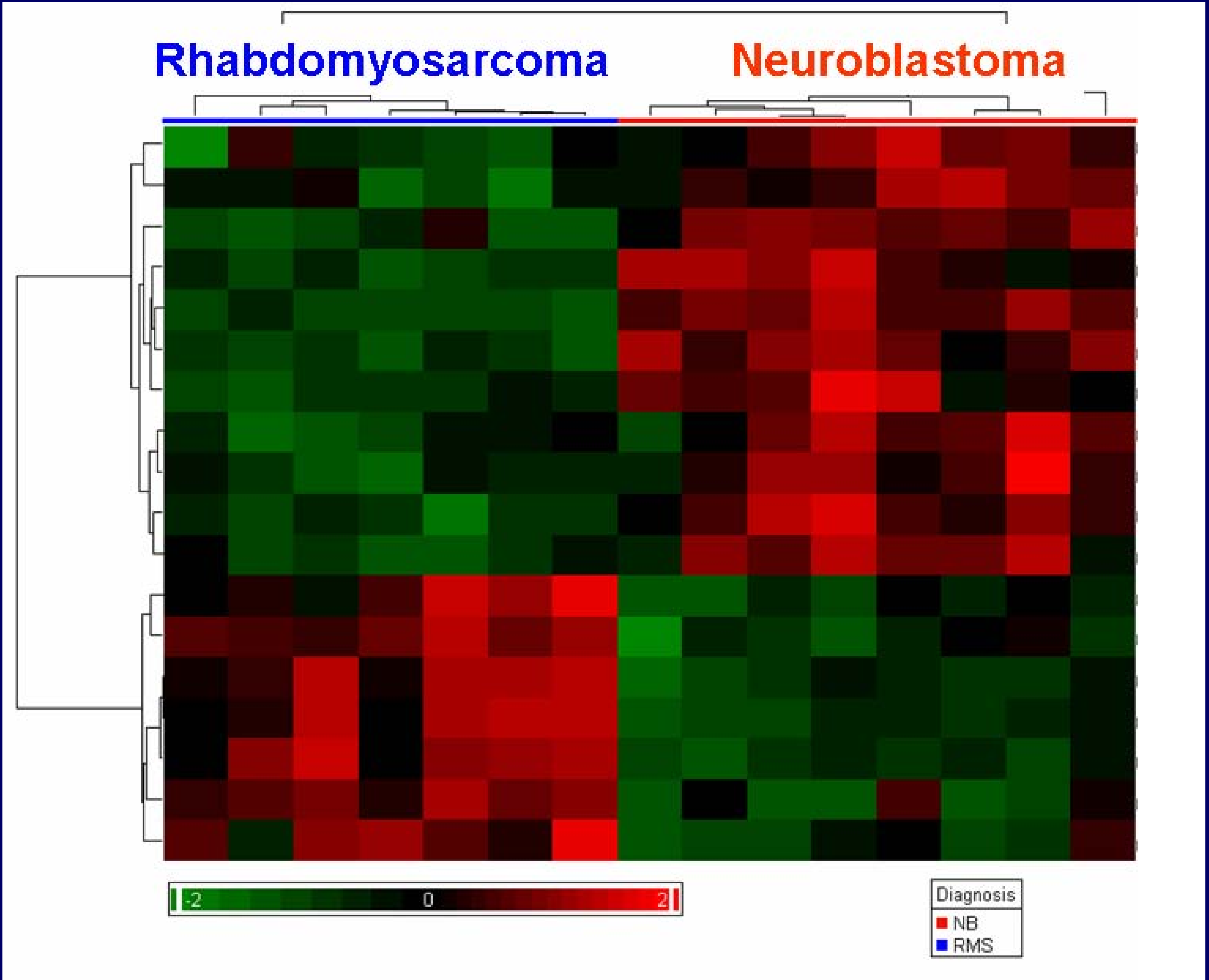
Hierarchical Clustering with All Probes



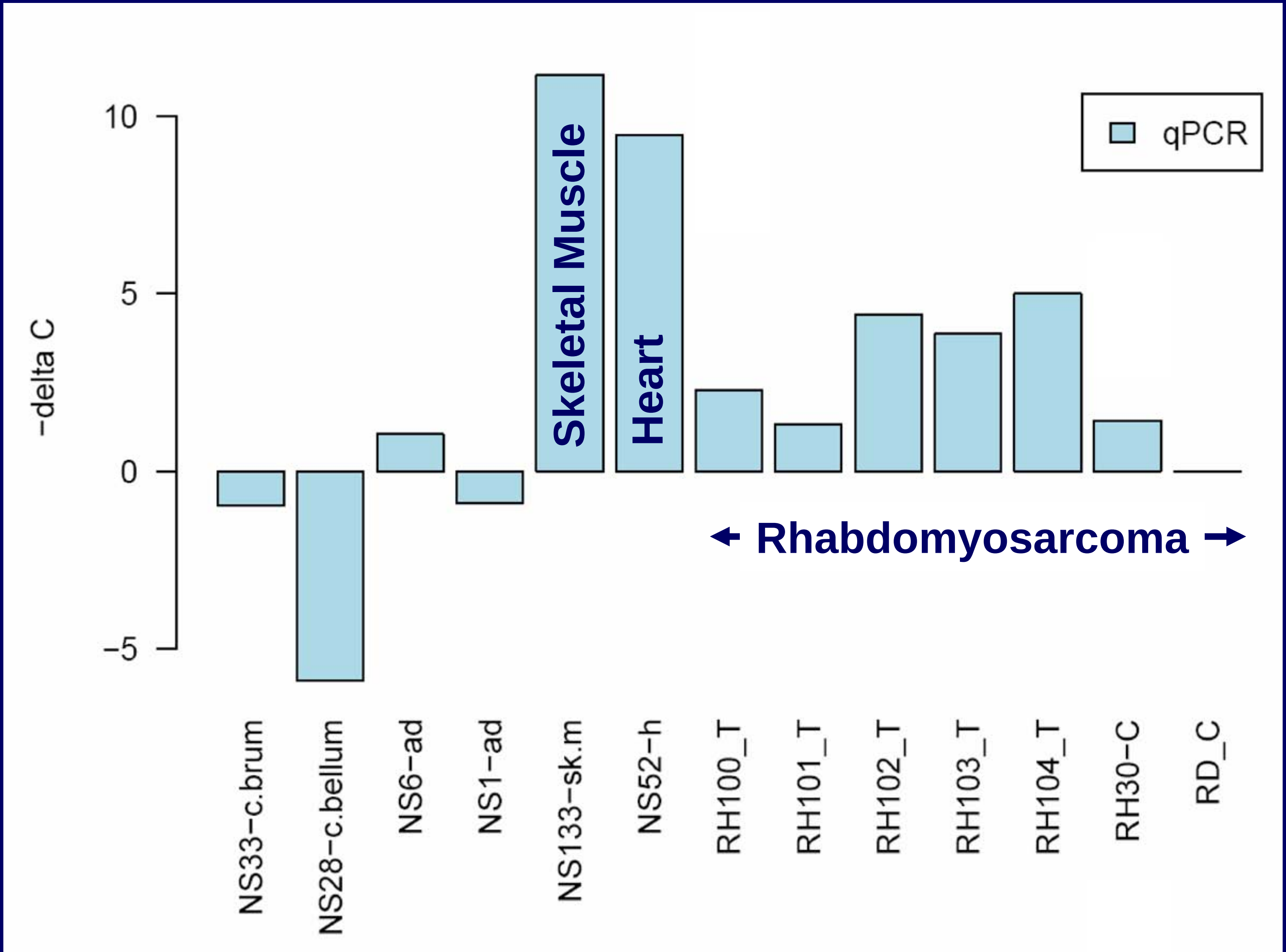
Hierarchical Clustering with All Probes



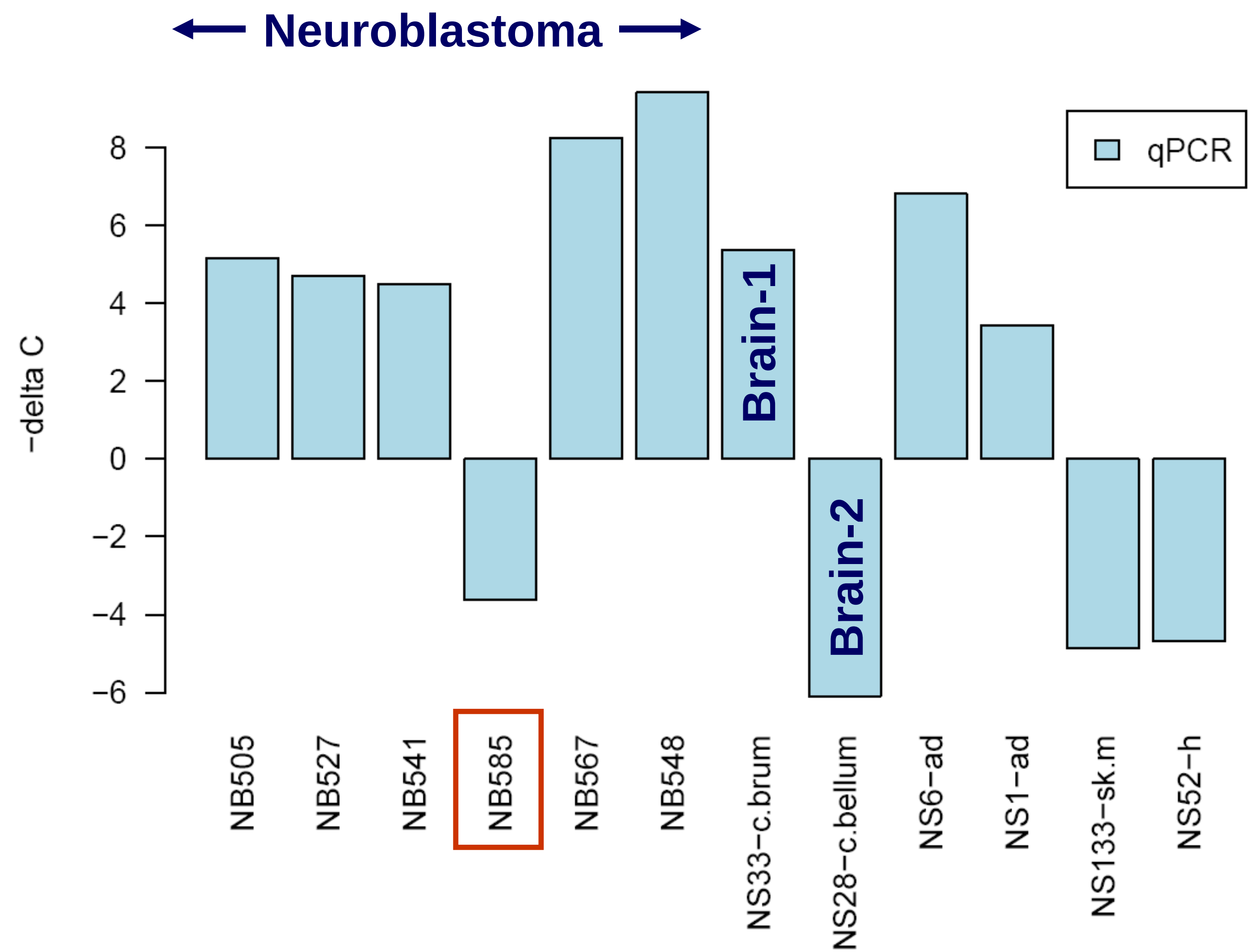
Cancer Specific miRNAs



miRNA is Expressed in RMS Tumors and Normal Muscles-Tissue Marker



miRNA is Expressed in NB Tumors and Normal Cerebrum



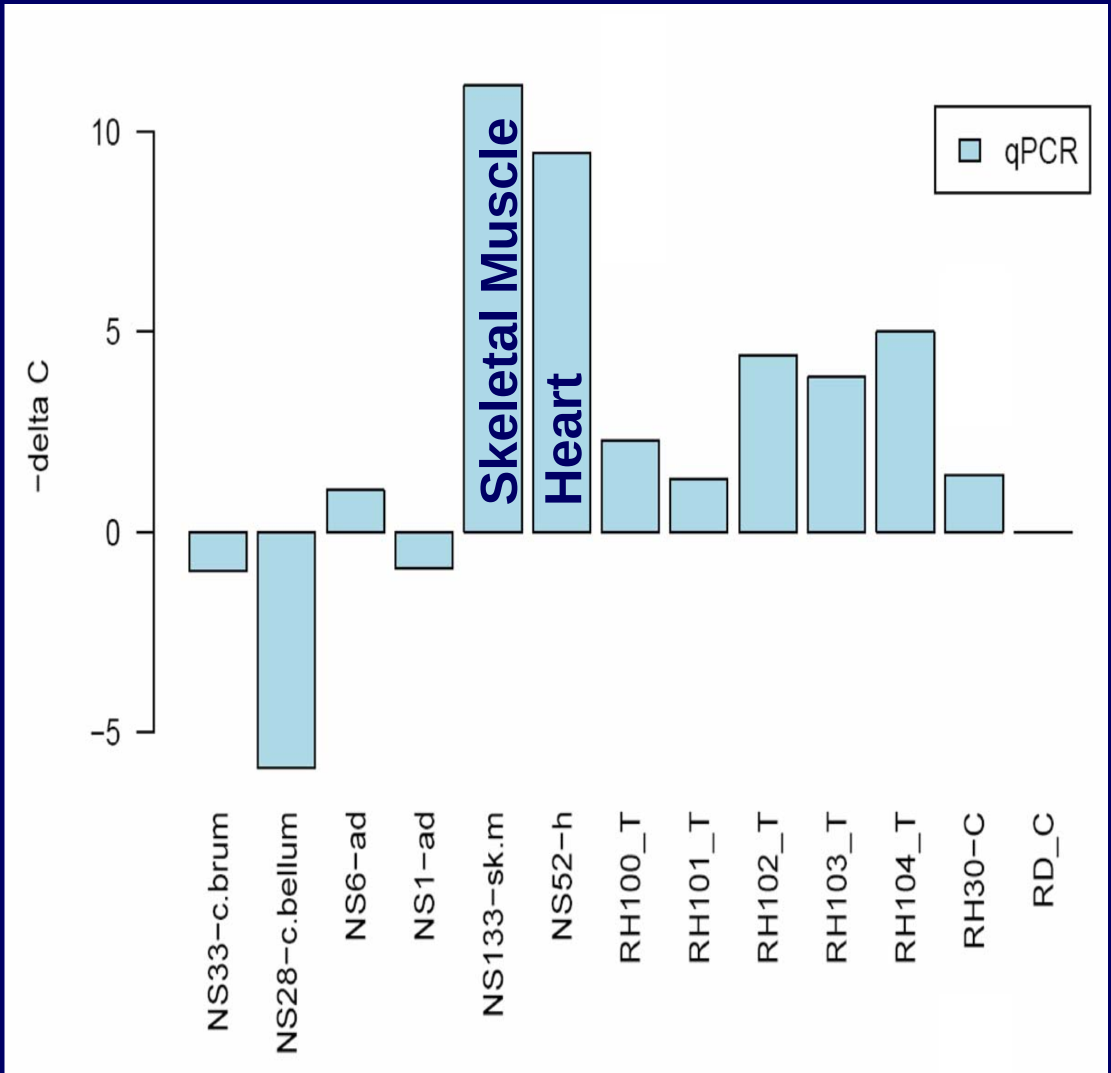
Goals for miRNA Profiling

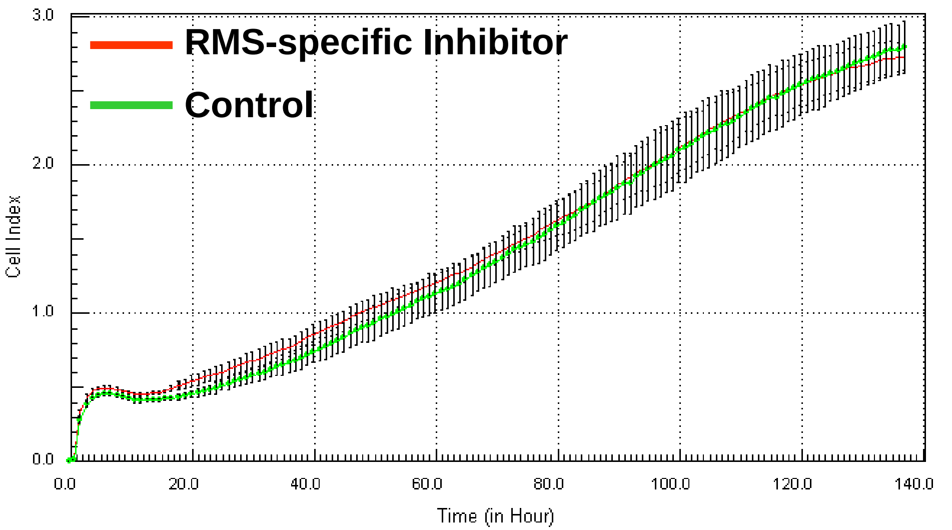
- Identify tumor-type-specific miRNAs
  - Tumor Classification
  - Diagnosis

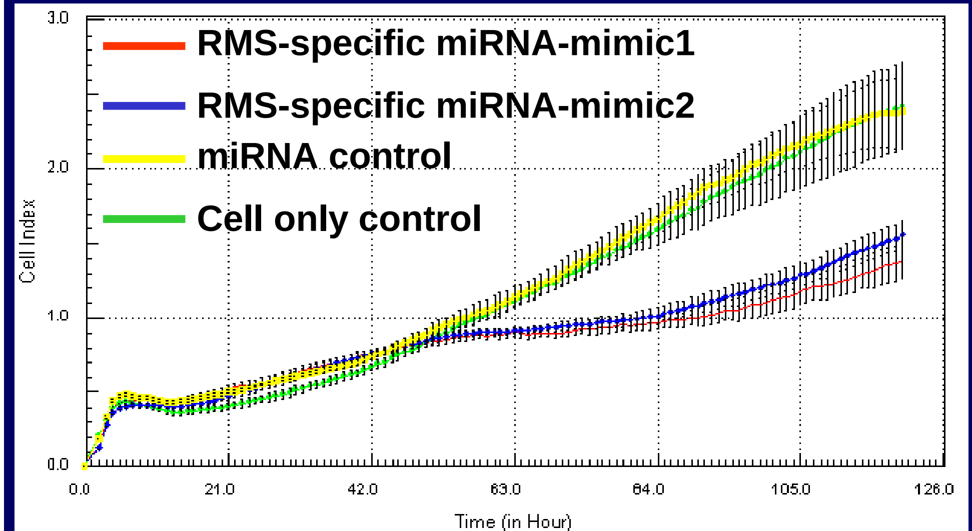
- Identify miRNA targets
  - Biology
  - Potential therapy

Function of mir in Rhabdomyosarcoma?

Suppresses Cell Growth







Conclusions

- microRNA have diverse biological function
- microRNA expression profiles reflects the tissue of origin of cancers and degree of differentiation
- May be useful for diagnosing cancers of unknown primaries
- May be useful for prognosis prediction
- May identify new targets for therapy (miRNA or anti miRNA)

However…..

- Classification performance not proven to be superior to messenger RNA profiling
- Multiple methods utilized for miRNA profiling
- microRNA extraction
  - Total- Includes Pri-miRNA, Pre-miRNA and mature miRNA
  - Size <200bp Includes Pre-miRNA and mature miRNA
  - Size <30bp- mature miRNA
- microRNA labeling
  - Poly A RNA polymerase-labels all fragments
  - PCR based-labels all RNA
- Platform
  - Beads- detects all
  - Home brew glass slide- detects all
  - Exiqon-detects all
  - Agilent-mature miRNA
  - RT-PCR-ABI-mature miRNA, other commercial-detects all
- Normalization methods—spiked in, tRNA, U4, U6…..

Future prospects…….. are good!

- Powerful new technologies: single molecule sequence based- profiling Illumina/Solexa etc
- New miRNA and other non-coding RNAs being discovered –may be better biomarkers/targets
- Standardization required-SOP
- Larger carefully designed studies with independent validation
- High potential high impact prospect for the future development of microRNA based diagnostic and prognostic biomarkers and therapeutic targets

Acknowledgements

Oncogenomics Section
Pediatric Oncology Branch, NCI

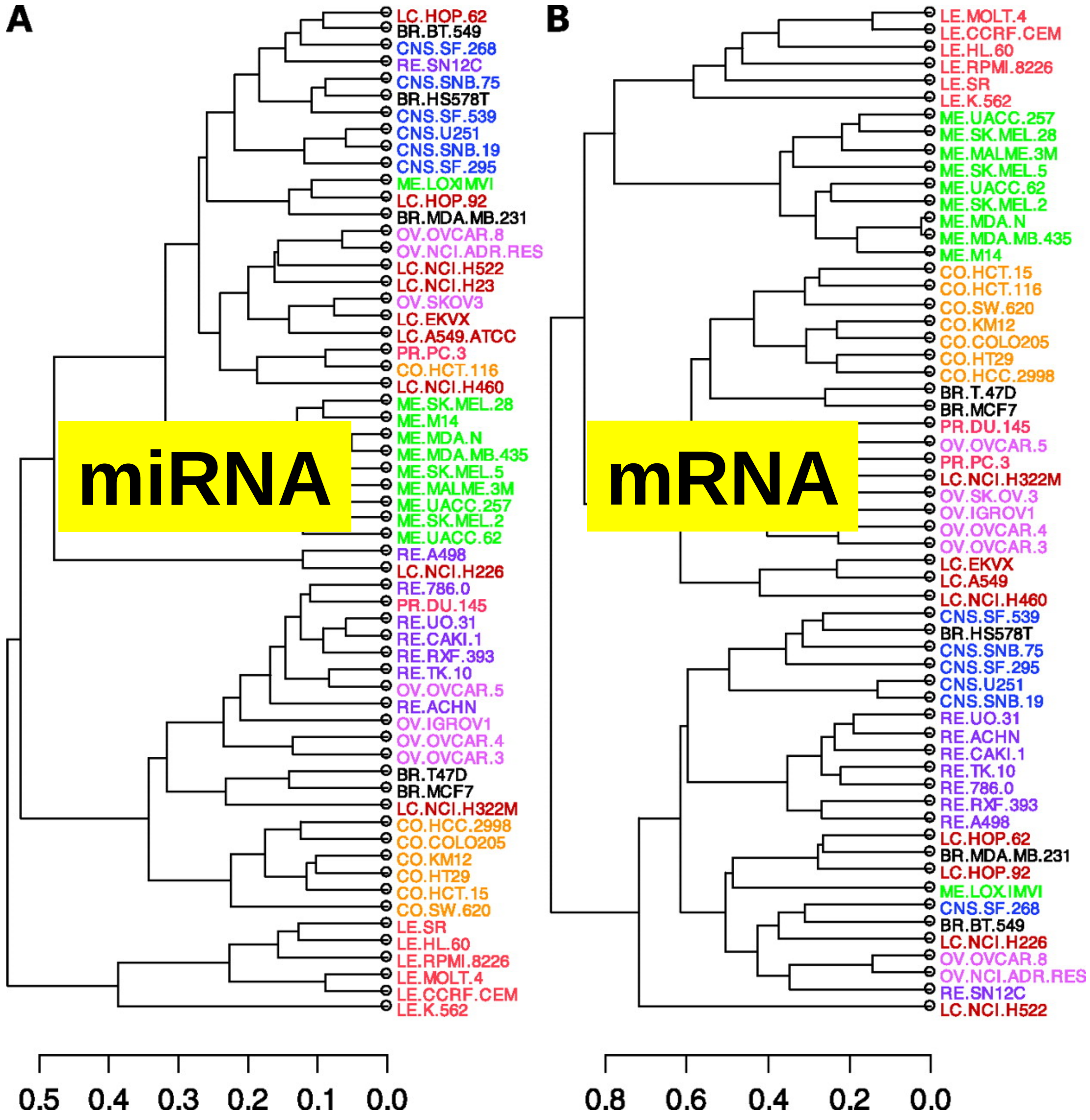
- Jun Wei
- Steffen Durinck
- Young Song
- Sven Bilke
- Qingrong Chen
- Craig Whiteford
- Braden Greer
- Nicola Cenacchi

Microarray Core Facility, NCI

- Ernest Kawasaki
- David Petersen
- Jonathon Paarlberg

Stephen Hewitt, TARP, NCI
Malcolm Smith, CTEP, NCI
Peter Houghton, St Judes

Parallel miRNA and mRNA profiling for NCI 60 Cell lines



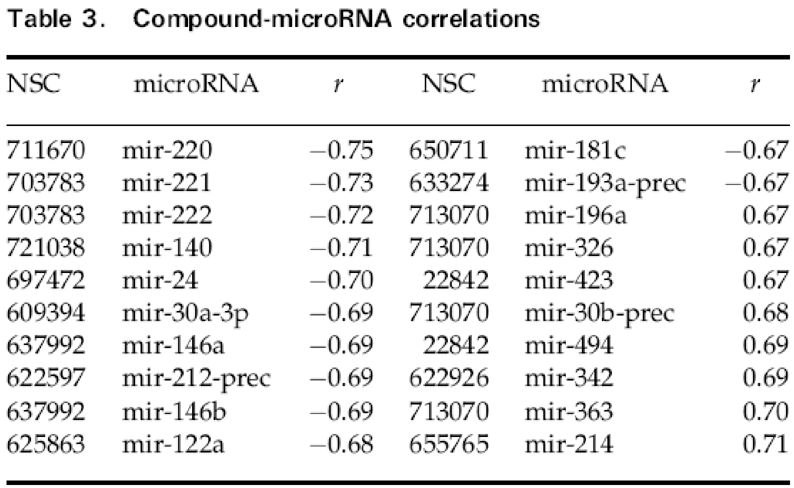


Table 3. Compound-microRNA correlations

NSC	microRNA	r	NSC	microRNA	r
711670	mir-220	−0.75	650711	mir-181c	−0.67
703783	mir-221	−0.73	633274	mir-193a-prec	−0.67
703783	mir-222	−0.72	713070	mir-196a	0.67
721038	mir-140	−0.71	713070	mir-326	0.67
697472	mir-24	−0.70	22842	mir-423	0.67
609394	mir-30a-3p	−0.69	713070	mir-30b-prec	0.68
637992	mir-146a	−0.69	22842	mir-494	0.69
622597	mir-212-prec	−0.69	622926	mir-342	0.69
637992	mir-146b	−0.69	713070	mir-363	0.70
625863	mir-122a	−0.68	655765	mir-214	0.71

- **mRNA expression more informative for discriminating among tissue types than was microRNA expression**
- **miRNA expression correlate with compound sensitivity**

Blower, P. E. et al. Mol Cancer Ther 2007;6:1483-1491