microRNAs (miRNA) and Biomarkers
“Small RNAs Make Big Splash”

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

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The Human Genome and Proteome

23 pairs of Chromosomes

3,000,000,000 bp

20-25,000 genes

>10,000 splice variants

>100,000 Proteins

>>10,000 non coding RNA

~1000 microRNA
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](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
1. miRNAs that bind with perfect or nearly perfect complementarity to protein coding mRNA sequences induce the RNA-mediated interference (RNAi) pathway causing mRNA degradation.

2. Imperfect complimentary binding to 3’-UTR results in translational repression.

3. Imperfect 3’-UTR binding 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

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

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

microRNA and Diagnosis

Microarray

microRNA

microRNA and Diagnosis

FDA Approves MammaPrint

van 't Veer LJ
Breast Cancer
Prognostic Signature
Nature 2002

MicroRNAs as Tumor Suppressors

MicroRNAs as Oncogenes

Result
Tumour formation
↑ Proliferation
↑ Invasion
↑ Angiogenesis
↓ Cell death

Esquela-Kerscher, et al.  
MicroRNA expression profiles classify human cancers


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

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
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

Identify Panel Similar to the Cancer of Origin

Tumors

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

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

Rhabdomyosarcoma  Neuroblastoma

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

- Rhabdomyosarcoma

[Diagram showing expression levels of miRNA in different tissues]
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
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mRNA expression more informative for discriminating among tissue types than was microRNA expression

miRNA expression correlate with compound sensitivity