**From the “one gene-one enzyme” hypothesis to…**

<table>
<thead>
<tr>
<th>DNA</th>
<th>RNA</th>
<th>microRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription factors</td>
<td>microRNA</td>
<td></td>
</tr>
</tbody>
</table>

**Seed**

5’-UGUGGCAAUCUAGCGAAACUGA-3’
(position 2 to 8)

**microRNA**

- Represent a class of small RNAs characterized by:
  - Short length: about 22 nucleotide-long
  - RNase III endonuclease-mediated processing
  - Association with Argonaute proteins
microRNA discovery

The C. elegans Heterochronic Gene lin-4 Encodes Small RNAs with Antisense Complementarity to lin-14

Hossein C. Lee, Thomas L. Hengst, and Victor Ambros
Department of Cell Biology and Developmental Biology
Cambridge, Massachusetts 02139

Posttranscriptional Regulation of the Heterochronic Gene lin-4 by lin-4 Mediates Temporal Pattern Formation in C. elegans

Bruce Wightman, Sara Ma, and Gary H. Hannon
Department of Molecular Biology
Massachusetts General Hospital
Boston, Massachusetts 02114

The discovery of microRNAs

- milestones -

1993 2000 2001
lin-4 discovery in C. elegans (Lee et al., Cell 1993; Hengst et al., Cell 1993)
let-7 discovery in H. sapiens (Pasquinelli et al., Nature 2000)
identification of a large class of small RNA called "microRNAs" (Lagos-Quintana et al., Lee et al., Lee and Ambros Science 2001)
microRNA biogenesis (Hutvagner et al., Science 2001; Lee et al., Nature 2003)
miRBase database (Griffith-Jones, NAR 2005; Griffith-Jones et al., NAR 2006)

microRNA biogenesis and function
**Canonical microRNA biogenesis**


**Substrate recognition by Rnase III endonucleases**

Microprocessor complex

(Drosha and DGCR8)


**Multifunctional microRNA precursors**

Non-canonical microRNA biogenesis

Drosha and DGCR8-independent

Canonical pathway

Dicer-independent

Possible mechanisms of microRNA-mediated repression

microRNA-mediated pathways
microRNA nomenclature

Precursor nomenclature:
- hsa-mir-19a
- hsa-mir-19b-1
- hsa-mir-19b-2

Mature nomenclature:
- hsa-miR-19a-3p
- hsa-miR-19a-5p
- hsa-miR-19b-1-5p
- hsa-miR-19b-2-5p
- hsa-miR-19b-3p

microRNA targets

microRNA-mediated target repression

Mature RISC

How do the mature RISC complexes recognize and bind their specific mRNA targets?
microRNA-target recognition - site position-

Binding in the 3'UTR is effective for repression

Effective sites in the 3'UTR are preferentially located in proximity of the termination codon (except the first 15 nt) or of the poly-A tail.


microRNA-target recognition -seed-

Canonical miRNA complementary sites

mRNA changes according to type and number of sites in the target 3'UTR


microRNA-target recognition -3' pairing-

Pairing in position 13-16 of the miRNA promotes target regulation

microRNA-target recognition - AU content -

Score changes based on the presence of AU relatively to the site

Effectiveness of sites with different local AU content


In summary, miRNAs bind to the 3'UTR of their mRNA targets according to the following rules:

- **site position** in proximity of the termination codon (except the first 15nt) or of the poly-A tail
- **perfect and contiguous base pairing** of miRNA nucleotides 2 to 8 (seed region)
- an A residue in position 1 of the site and/or 3' complementarity especially at position 13-16 of the miRNA improve the site efficiency
- bulges or mismatches must be present in the middle of the miRNA-mRNA duplex, precluding the AGO2-mediated cleavage of mRNA
- AU-rich neighborhoods host more effective sites

Identification of microRNA targets

**Target Prediction Algorithms**
- TargetScan
- PicTar
- MiRanda
- PIYA
- ...many others

**Cell context specificity**
- Gene Expression Profiles
- Differential Expression
- Proteomic Profiles

**Experimental Validation**
- 3'UTR-reporter assay
- AGO-IP
- CLIP (Cross-Link and ImmunoPrecipitation)
**microRNA-mediated networks**

**The “competing endogenous RNA” (ceRNA) hypothesis**

“All types of RNA transcripts communicate through a “new” language mediated by miRNA response elements”


Effects of microRNA-target perturbation

**Competing target overexpression**

**Steady-state**

**Competing miRNA overexpression**
microRNA-mediated modulation of PTEN

Discovery of miR-15 and miR-16 as onco-suppressors in Chronic Lymphocytic Leukemia (Calin et al., PNAS 2002)

Oncomir-1 (miR-17-92 cluster) (Hu et al., Nature 2005; O'Donnell et al., Nature 2005)

miRNA profiling classifies tumors (Lu et al., Nature 2005)

microRNA & metastasis (Ma et al., Nature 2007; Tavazoie et al., Nature 2008)

miRNA detection in serum and plasma (Chen et al., Cell Res. 2008; Mitchell et al., PNAS 2008)

microRNAs and cancer

microRNAs and cancer research -milestones-
microRNAs in cancer

- Normal tissue
- MicroRNA functioning as tumor suppressor
  - Decreased miRNA expression
  - Enhanced target repression
- MicroRNA functioning as oncogene
  - Increased miRNA expression
  - Enhanced target repression

Genetic, transcriptional and post-transcriptional microRNA alterations in cancer

Genomic Alterations
- Amplification
- Deletion
- Mutation

Transcription & Processing
- Target modifications

Genomic Alterations
- Amplification
- Deletion
- Mutation

MicroRNA deregulated activity induced by target modifications

Genomic Alterations
- Amplification
- Deletion
Pathways leading to altered microRNA expression and/or activity

- Genomic lesions (gains, losses, rearrangements)
- Deregulated transcription
- Mutations/SNPs affecting miRNA and/or its processing

microRNAs as tumor suppressors

Chronic Lymphocytic Leukemia (CLL)

- CLL represents the most frequent B-cell malignancy in the elderly
- Characteristically expresses the CD5 cell surface antigen
- It can be divided into cases with somatically mutated (~60%) or unmutated (~40%) immunoglobulin variable genes
Recurrent genetic aberrations in CLL

- Trisomy 12 (16%)
  - is thought to affect gene dosage of unknown genes

- Deletion of 11q (18%)
  - deletion of the ATM gene; may predispose to genomic instability and the development of lymphoid malignancy

- Deletion of 17q (7%)
  - deletion of the TP53 gene, and in most cases, the remaining TP53 allele is mutated; predisposes to genomic instability and the development of lymphoid malignancy

- Deletion of 13q14 (55%)
  - ?

The 13q14 Minimal Deleted Region (MDR) contains multiple genetic elements

Mouse model of the 13q14 deletion

Mouse model #1
MDR conditional knock-out
Mouse model #2
mir-15a/16-1 conditional knock-out
RT-PCR for dLeu2

Mouse 14qC3
14qC3
Mouse model #1
MDR conditional knock-out

Human 13q14
13q14
13q14
KO
Mir-15a/16-1
DLEU2
DLEU1

Mouse model #2
mir-15a/16-1 conditional knock-out

RT-PCR for dLeu2

Mouse model #1
MDR conditional knock-out

KO
Mir-15a/16-1
DLEU2
DLEU1

Mouse model #2
mir-15a/16-1 conditional knock-out

RT-PCR for dLeu2

Mouse model #1
MDR conditional knock-out

KO
Mir-15a/16-1
DLEU2
DLEU1

Mouse model #2
mir-15a/16-1 conditional knock-out

RT-PCR for dLeu2

Mouse model #1
MDR conditional knock-out

KO
Mir-15a/16-1
DLEU2
DLEU1

Mouse model #2
mir-15a/16-1 conditional knock-out

RT-PCR for dLeu2
Malignancies in mouse models of the 13q14 deletion

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>MDR- and mir-15a/16-1-deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid involvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonal Ig</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Spleen (H&amp;E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Blood (FACS)</td>
<td>12%</td>
<td>12%</td>
</tr>
<tr>
<td>CD5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B220</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>


Malignancies in mouse models of the 13q14 deletion - low penetrance and indolent phenotype -

- mir-15a/16-1
- MDR


microRNAs and host genes: synergistic and antagonistic functions

### miR-15a and miR-16 modulate cellular proliferation

<table>
<thead>
<tr>
<th>Protein</th>
<th>miR-15a/16-1</th>
<th>miR-15a/16-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL-515E5E</td>
<td>+2.2</td>
<td>+2.4</td>
</tr>
<tr>
<td>CCNE1</td>
<td>+2.0</td>
<td>+5.4</td>
</tr>
<tr>
<td>CCNE2</td>
<td>+3.8</td>
<td>+3.5</td>
</tr>
<tr>
<td>CDK4</td>
<td>+1.7</td>
<td>+1.7</td>
</tr>
<tr>
<td>Rb</td>
<td>-4.9</td>
<td>-4.9</td>
</tr>
</tbody>
</table>

### microRNAs as oncogenes

- **miR-15a** and **miR-16** modulate cellular proliferation

### 13q31-q32 amplification in B cell lymphoma

- Searching for candidate oncogene(s)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Sample</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q31-q32</td>
<td>Karpas1818 (BLCL)</td>
<td>Ch13: G0/G1</td>
</tr>
<tr>
<td></td>
<td>Rec1 (MCL)</td>
<td>Ch13: G0/G1</td>
</tr>
<tr>
<td></td>
<td>OCL-Ly4 (DLBCL)</td>
<td>Ch13: G0/G1</td>
</tr>
<tr>
<td></td>
<td>Jurkat (T-ALL)</td>
<td>Ch13: G0/G1</td>
</tr>
</tbody>
</table>
mir-17-92 cluster on chr 13q31-q32 and its paralogues


mir-17-92 cluster: oncomir-1

Acceleration of MYC-induced lymphomagenesis in mice


Over-expression in cancer

mir-17-92 cluster: function and regulation

**MicroRNAs in cancer**

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Expression in patients</th>
<th>Confirmed targets</th>
<th>Experimental data</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15a</td>
<td>downregulated in lung and breast cancers</td>
<td>RAS, c-Myc, MYCN</td>
<td>Patient survival</td>
<td>TS</td>
</tr>
<tr>
<td>miR-16-1</td>
<td>downregulated in CLL, AML, FL</td>
<td>Bcl-2, Wt-1</td>
<td>Decrease tumorigenicity</td>
<td>TS</td>
</tr>
<tr>
<td>miR-17-5p</td>
<td>downregulated in lymphomas, lung and breast cancers</td>
<td>TGF-β, TGF-βR</td>
<td>Induce apoptosis, decrease tumorigenicity</td>
<td>TS</td>
</tr>
<tr>
<td>miR-17-92 cluster</td>
<td>upregulated in lymphomas and in breast, lung, colon, stomach, and pancreas cancers</td>
<td>E2F1, Bim, PTEN</td>
<td>Cooperates with c-Myc to induce lymphoma in mice, transgenic miR-17-92 develop lymphoproliferative disorder</td>
<td>OG</td>
</tr>
<tr>
<td>miR-21</td>
<td>upregulated in breast, colon, pancreas, lung, prostate, liver, and stomach cancer; AML (11q23), LL, and glioblastoma</td>
<td>PTEN, PDCD4, TPM1</td>
<td>Decrease tumorigenicity</td>
<td>DG</td>
</tr>
<tr>
<td>miR-372/miR-373</td>
<td>upregulated in testicular tumors</td>
<td>LATS2</td>
<td>Decrease tumorigenicity in cooperation with RAG</td>
<td>DG</td>
</tr>
</tbody>
</table>

**MicroRNA-mediated target repression**

- **Summary**
  - pri-miRNA
  - pre-miRNA
  - mature miRNA
  - Target
  - miRNA-mediated target repression
  - Translational repression
  - mRNA degradation