Role of microRNAs in Human Diseases

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The genetic basis of developmental complexity

- Humans (and other vertebrates) have approximately the same number of protein-coding genes (~20,000) as *C. elegans*, and less than those of plants (*Arabidopsis* ~28,000, rice ~40,000) and protozoa (30,000).

- Most of the proteins are orthologous and have similar functions from nematodes to humans, and many are common with yeast.

→ Where is the information that programs our complexity?
The proportion of noncoding DNA broadly increases with developmental complexity.
Type of RNA molecules

- **RNA**
  - **mRNA**
    - Protein-coding RNA
  - **ncRNA: non-coding RNAs**
    - Transcribed RNA with a structural, functional or catalytic role
      - **rRNA**
        - Ribosomal RNA
        - Participate in protein synthesis
      - **tRNA**
        - Transfer RNA
        - Interface between mRNA & amino acids
      - **snRNA**
        - Small nuclear RNA
        - RNA that form part of the spliceosome
      - **snoRNA**
        - Small nucleolar RNA
        - Found in nucleolus, involved in modification of rRNA
      - **RNAi**
        - RNA interference
        - Small non-coding RNA involved in regulation of gene expression
      - **siRNA**
        - Small interfering RNA
        - Active molecules in RNA interference
      - **miRNA**
        - MicroRNA
        - Small RNA involved in regulation of protein-coding gene
      - **Other**
        - Including large RNA with roles in chromatin structure and imprinting
Non-coding RNAs challenge the central dogma of biology

Conventional views

- 6.4 billion bases in diploid cells
- 25,000 protein-coding genes (2% of the genome)
- ~60,000 proteins (alternative splicing, PTM)
- Only regulated by protein coding genes

Current model

- 6.4 billion bases in diploid cells
- 25,000 coding genes
- Non-coding RNAs (>60% of the genome)
- ~60,000 proteins
- Many non-coding RNAs
- Regulated by proteins and non-coding RNAs
C. elegans lin-4: first identified microRNA

- lin-4 encodes two small RNA molecules, a more abundant 22 nt that are processed from a rare 61 nt pre-lin-4. These hairpin precursor is a characteristic feature of the miRNA class of regulatory RNAs.

- One of lin-4’s target genes, lin-14, encodes a novel nuclear protein and is a putative transcription factor. The lin-4 microRNA regulates lin-14 through specific sequences in the 3’ UTR of the lin-14 mRNA.

- Upon lin-4 expression, lin-14 protein levels are reduced. Although transcription from the lin-14 gene still occurs, it is of no consequence. (Posttranscriptional control).
lin-4 and let-7 are funding members of microRNA

- Seven years later, let-7 (another non-coding gene) was shown to regulate development in worms
- A homolog of let-7 was identified in humans and Drosophila
- Lin-4 and let-7 became founding members of a group of endogenous small RNA molecules with regulatory functions

Lin-4: regulates heterochronic development at L1 to L2 stage
Let-7: regulates heterochronic development at L4 to adult stage

Let-7 sequence and gene regulation microRNA binding sites in the 3' UTR region also showed evolutional conservation
microRNAs at a glance

- Small, single-stranded forms of RNA (~22 nucleotides in length)
- Generated from endogenous hairpin-shaped transcripts encoded in the genomes
- Negatively regulate protein-coding genes through translational repression or targeting mRNA for degradation
- More than 1,000 microRNAs encoded in human genome constitute a largest gene family
- It has been estimate that more than 30% of protein-coding genes can be regulated by microRNAs
More than 15,000 miRNAs in public databases

- Homo sapiens (1048)
- Mus musculus (672)
- Rattus norvegicus (408)
- Drosophila melanogaster (176)
- Caenorhabditis elegans (175)
- Arabidopsis thaliana (213)
- Epstein Barr virus (25)
- Human cytomegalovirus (11)
- Kaposi sarcoma-associated herpesvirus (13)
- Simian virus 40 (1)

From miRBase Release 16 (Sept. 2010)

More than 1200 mature miRNAs in human genome
microRNA Nomenclature

- **Pri-miR-1; Pre-miR-1; miR-1**: type of microRNA
- **miR-10a; miR-10b**: microRNA family
- **miR-9-1; miR-9-2; miR-9-3**: same mature miRNA derived from different genome locus
- **miR-127-3p; miR-127-5p**: different mature miRNAs derived from same precursor
- **miR-96; miR-96**: start denote the less abundant mature miRNA

**miRNA family**

- Let-7a (Chr9) UGAGGUAGUAGGUUGUAUAGUU
- Let-7b (Chr22) UGAGGUAGUAGGUUGUGGUU
- Let-7c (Chr21) UGAGGUAGUAGGUUGUAUGUU
- Let-7d (Chr9) AGAGGUAGUAGGUUGCAGUAGUU
- Let-7e (Chr19) UGAGGUAGGAAGGUAUAGUU
- Let-7f (Chr9) UGAGGUAGUAGAUUGUAUAGUU
- Let-7g (Chr3) UGAGGUAGUGUUGUAAGUU

**Same miRNA from different locus**

- **Hsa-miR-9-1 (Chr 1)** CGGGGUUGGUUGUUAUCUUUGGUUAUCAGCUGUAUGAGUGGUGUUGAGUCUUCAUAAAGCUAGAUAAACCGAAAGUAAGAAUAACCCCCCA
- **Hsa-miR-9-2 (Chr 5)** GGAAGCGAGUUGUUAUCUUUGGUUAUCAGCUGUAUGAGUGAUUGGUCUUCAUAAAGCUAGAUAAACCGAAAGUAAGAAUAACCUCCUCA
- **Hsa-miR-9-3 (Chr 15)** GGAGGCCGUUUCUCUCUUGGUUAUCAGCUGUAUGAGUGCCACAGAGCUGUCAUAAAGCUAGAUAAACCGAAAGUAAGAAUAACCUCCUCA
microRNA biogenesis

Transcription

Processing

Maturation

Execution

Nature Rev. Immunology (2008) 8: 120-130
miRNA-mediated gene silencing

In animal cells:
1. Translational repression
2. mRNA destabilization
Gene regulation by transcription factors and microRNAs

<table>
<thead>
<tr>
<th>Transcription factors</th>
<th>microRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleiotropy</td>
<td></td>
</tr>
<tr>
<td>Target #1</td>
<td>Target #1</td>
</tr>
<tr>
<td>Target #2</td>
<td>Target #2</td>
</tr>
<tr>
<td>Target #3</td>
<td>Target #3</td>
</tr>
<tr>
<td>Combinatorial and cooperative activity</td>
<td></td>
</tr>
<tr>
<td>Cell type #1</td>
<td>Cell type #2</td>
</tr>
<tr>
<td>Target #1</td>
<td>Target #2</td>
</tr>
<tr>
<td>Accessibility</td>
<td></td>
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<tr>
<td>Nucleosomes</td>
<td>mRNPs and/or Secondary structure</td>
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<tr>
<td>Regulation</td>
<td></td>
</tr>
<tr>
<td>Processing</td>
<td>Processing</td>
</tr>
<tr>
<td>Modification</td>
<td>Editing</td>
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<tr>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>Network motifs</td>
<td></td>
</tr>
<tr>
<td>Feedforward loop</td>
<td></td>
</tr>
</tbody>
</table>

Example: feedforward loop
microRNA networks

- The number of microRNA in human genome may over 1,000 genes (currently 970 mature miRNA sequences in miRBase database)

- Tens to hundreds of protein-coding genes are regulated by single miRNA

- Estimated that around 30% to 50% of protein coding genes are regulated by microRNA

- Almost every cellular processes are regulated by microRNA

Mutation or alteration of microRNA networks will cause human diseases
microRNA associated human diseases

- Human malignancies
  - Leukemia/lymphoma
  - Solid tumor: breast cancer, colon cancer, lung cancer, brain tumor

- Heart diseases
  - cardiac hypertrophy, cardiac arrhythmias,
  - heart failure, congenital heart disease
  - muscular dystrophy

- Immunological disorders

- Stem cell differentiation and development
microRNA and Human Malignancies
Several evidences suggest that microRNAs may play an important role in tumor development

- More than 50% of microRNAs are located within the chromosome fragile sites
- Expression levels of microRNA in tumor biopsies are commonly altered
- microRNAs play an important role in the regulating cell proliferation, differentiation and cell survival.
- Several microRNAs have been shown to function as oncogenes or tumor suppressors
- microRNAs also regulate tumor angiogenesis and metastasis
miRNA frequently located at chromosome fragile sites
# Examples of miRNAs located in chromosome fragile sites

Around 50% of microRNAs are located in chromosome fragile sites, why?

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Location (defining markers)</th>
<th>Size (Mb)</th>
<th>MiR</th>
<th>Hystotype</th>
<th>OG or TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3p21.1-21.2-D</td>
<td>ARP-DRR1</td>
<td>7</td>
<td>Let-7g and miR-135-1</td>
<td>Lung and breast cancer</td>
<td>Not known</td>
</tr>
<tr>
<td>3p21.3(AP20)-D</td>
<td>GOLGA4-VILL</td>
<td>0.75</td>
<td>MiR-26a</td>
<td>Epithelial cancer</td>
<td>Not known</td>
</tr>
<tr>
<td>3p23-21.31(MDR2)-D</td>
<td>D3S1768-D3S1767</td>
<td>12.32</td>
<td>Mir-26a and miR-138-1</td>
<td>Nasopharyngeal cancer</td>
<td>Not known</td>
</tr>
<tr>
<td>5q32-D</td>
<td>ADRB2-ATX1</td>
<td>2.92</td>
<td>MiR-145 and miR-143</td>
<td>Myelodysplastic syndrome</td>
<td>Not known</td>
</tr>
<tr>
<td>9q22.3-D</td>
<td>D9S280-D9S1809</td>
<td>1.46</td>
<td>MiR-24-1, miR-27b and miR-23b; let-7a-1, let-7f-1 and let-7d</td>
<td>Urothelial cancer</td>
<td>PTC and FANCC</td>
</tr>
<tr>
<td>9q33-D</td>
<td>D9S1826-D9S158</td>
<td>0.4</td>
<td>MiR-123</td>
<td>NSCLC</td>
<td>Not known</td>
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<tr>
<td>11q23-q24-D</td>
<td>D11S927-D11S1347</td>
<td>1.994</td>
<td>MiR-34a-1 and miR-34a-2</td>
<td>Breast and lung cancer</td>
<td>PPP2R1B</td>
</tr>
<tr>
<td>11q23-q24-D</td>
<td>D11S1345-D11S1328</td>
<td>1.725</td>
<td>MiR-125b-1, let-7a-2 and miR-100</td>
<td>Breast, lung, ovary and cervix cancer</td>
<td>Not known</td>
</tr>
<tr>
<td>13q14.3-D</td>
<td>D13S272-D13S25</td>
<td>0.54</td>
<td>MiR-15a and miR-16a</td>
<td>B-cell CLL</td>
<td>Not known</td>
</tr>
<tr>
<td>13q32-33-A</td>
<td>stSG15303-stSG31624</td>
<td>7.15</td>
<td>MiR-17, miR-18, miR-19a, miR-20, miR-19b-1 and miR-92-1</td>
<td>Follicular lymphoma</td>
<td>Not known</td>
</tr>
<tr>
<td>17p13.3-D</td>
<td>D17S1866-D17S1574</td>
<td>1.899</td>
<td>MiR-22, miR-132 and miR-212</td>
<td>HCC</td>
<td>Not known</td>
</tr>
<tr>
<td>17p13.3-D</td>
<td>ENO3-TP53</td>
<td>2.275</td>
<td>MiR-195</td>
<td>Lung cancer</td>
<td>TP53</td>
</tr>
<tr>
<td>17q22-21(8;17)</td>
<td>MiR-142s-c-MYC</td>
<td>MiR-142s and miR-142as</td>
<td>Prolymphocytic leukemia</td>
<td>c-MYC</td>
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</tr>
<tr>
<td>17q23-A</td>
<td>CLTC-PPM1D</td>
<td>0.97</td>
<td>MiR-21</td>
<td>Neuroblastoma</td>
<td>Not known</td>
</tr>
<tr>
<td>20q13A</td>
<td>FLJ38887-ZNF217</td>
<td>0.55</td>
<td>MiR-297-3</td>
<td>Colon cancer</td>
<td>Not known</td>
</tr>
<tr>
<td>21q11.1-D</td>
<td>D21S1911-ANA</td>
<td>2.84</td>
<td>MiR-99a, let-7c and miR-125b</td>
<td>Lung cancer</td>
<td>Not known</td>
</tr>
</tbody>
</table>

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**D**: deleted region  
**A**: amplified region

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This Table was reproduced, with permission, from Ref. [13].

CLL, chronic lymphocytic leukemia; FANCC, Fanconi anemia, complementation group C; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung carcinoma; OG, oncogene; PPP2R1B, protein phosphatase 2, regulatory subunit A, β isoform; PTC, papillary thyroid carcinoma; TP53, tumor protein 53; TS, tumor suppressor.
microRNAs are commonly down regulated in tumor biopsies
microRNA expression pattern can differentiates tumor from normal tissues

- miRNA down-regulated in tumor tissues
  (tumor suppressive miRNAs?)

- miRNA up-regulated in tumor tissues
  (Oncogenic miRNAs?)
Acquisition of tumorigenic phenotype by multiple mutations

Mutation on oncogenes and tumor suppressors

- Cell acquires properties of neoplastic transformation.
  - Growth is: Unregulated, Anchorage independent, Non-contact inhibited
- Tumour formation: All cells have identical genotype
Examples of Oncogenes

Common actions of oncogene: cell proliferation and anti-apoptosis

- Transcription factors: c-my, c-Jun, c-Fos
- Receptor tyrosine kinases: erbB2, EGFR, PDGFR, VEGFR
- Cytoplasmic tyrosine kinase: Src, Abl
- Small G proteins: Ras
- Ser/Thr kinases: Raf, MAPK, CDK

Table 1  Examples of human oncogenes

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Originally identified in</th>
<th>Mechanism of activation in human tumours</th>
<th>Location</th>
<th>Associated human cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>src</td>
<td>Rous sarcoma virus</td>
<td>Overexpression, C-terminal deletion</td>
<td>Cytoplasmic</td>
<td>Breast, colon, lung carcinomas</td>
</tr>
<tr>
<td>myc</td>
<td>Avian myelocytomatosis virus</td>
<td>Translocation</td>
<td>Nuclear</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>abl</td>
<td>Abelson murine leukaemia virus</td>
<td>Translocation</td>
<td>Cytoplasmic</td>
<td>Chronic myeloid leukaemia</td>
</tr>
<tr>
<td>Ha-ras</td>
<td>Harvey murine sarcoma virus</td>
<td>Point mutation</td>
<td>Cytoplasmic</td>
<td>Bladder cancer</td>
</tr>
<tr>
<td>K-ras</td>
<td>Kirsten murine sarcoma virus</td>
<td>Point mutation</td>
<td>Cytoplasmic</td>
<td>Colon, lung carcinomas</td>
</tr>
<tr>
<td>erbB (EGFR)</td>
<td>Avian erythroblastosis virus</td>
<td>Overexpression, deletion</td>
<td>Cytoplasmic</td>
<td>Breast carcinoma, glioblastoma</td>
</tr>
</tbody>
</table>
Tumor suppressor genes

A tumor suppressor gene is a gene that protects a cell from one step on the path to cancer. When this gene is damaged, the cell can progress to cancer.

Table 2: Tumour-suppressor genes and their function and associated cancers

<table>
<thead>
<tr>
<th>Name</th>
<th>Function in normal cells</th>
<th>Associated cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Cell cycle regulator</td>
<td>Colon and others</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Cell cycle regulator, genomic integrity and chromatin structure</td>
<td>Breast, ovarian, prostate and others</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Genomic integrity</td>
<td>Breast, ovarian, prostate and others</td>
</tr>
<tr>
<td>PTEN</td>
<td>Tyrosine and lipid phosphatase</td>
<td>Prostate, glioblastomas</td>
</tr>
<tr>
<td>APC</td>
<td>Cell adhesion</td>
<td>Colon</td>
</tr>
<tr>
<td>DCC</td>
<td>Cell adhesion</td>
<td>Colon</td>
</tr>
<tr>
<td>MCC</td>
<td>Undetermined</td>
<td>Colon</td>
</tr>
<tr>
<td>p16-INK4A</td>
<td>Cell cycle regulator</td>
<td>Colon and others</td>
</tr>
<tr>
<td>MLH1</td>
<td>Mismatch repair</td>
<td>Colon and gastric cancers</td>
</tr>
<tr>
<td>MSH2</td>
<td>Mismatch repair</td>
<td>Colon and gastric cancers</td>
</tr>
<tr>
<td>DPC4</td>
<td>Cell death regulator</td>
<td>Pancreatic</td>
</tr>
<tr>
<td>Wt1</td>
<td>Cell death regulator</td>
<td>Wilms’ tumour</td>
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<tr>
<td>NF1</td>
<td>Regulator of GTPases</td>
<td>Astrocytomas</td>
</tr>
<tr>
<td>NF2</td>
<td>Cell adhesion</td>
<td>Astrocytomas</td>
</tr>
<tr>
<td>VHL</td>
<td>Ubiquitination</td>
<td>Renal</td>
</tr>
<tr>
<td>PTC</td>
<td>Regulator of hedgehog signalling</td>
<td>Thyroid</td>
</tr>
<tr>
<td>TSC2</td>
<td>Cell cycle regulator</td>
<td>Breast and renal</td>
</tr>
<tr>
<td>TSG101</td>
<td>Cell cycle regulator</td>
<td>Renal and leukaemia</td>
</tr>
</tbody>
</table>
microRNAs can function as oncogenes or tumor suppressors

a) Normal tissues

b) MicroRNA functioning as a tumour suppressor

c) MicroRNA functioning as an oncogene

Result
Normal rate of growth, proliferation, differentiation and cell death

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

Result
Tumour suppression
↓ Proliferation
↓ Invasion
↓ Angiogenesis
miR-17/92 cluster showed increase expression in B lymphoma and colon cancers
miR-17/92 clusters function as oncogenes

- Overexpression of the mir-17-19b cluster accelerates c-myc-induced lymphomagenesis in mice

- Em-myc/mir-17-19b tumors show a more disseminated phenotype compared with control tumor

miR-34 family function as tumor suppressors

- miR-34 family members are highly conserved during evolution
- miR-34a is located within chromosome 1p36 region, which is commonly deleted in human neuroblastoma
- Primary neuroblastomas and cell lines often showed low levels of miR-34a expression
- Forced expression of miR-34a in these cells inhibited proliferation and activated cell death pathways
Extensive vascularization in solid tumors

Angiogenesis in solid tumor
• Provide nutrients for cell growth
• Support oxygen for energy production
• Remove cellular metabolites (Waste)
Angiogenesis: a key transition in tumor development

- Initiation
- Promotion
- Dormant in situ
- Cancer
- Established tumor
- Metastasis

Accessory cells

Angiogenic switch

1 kg
1 g
1 mg
1 μg
1 ng
Expression of miR-378 Promotes Tumorigenesis and Angiogenesis

A. Capillary formation

B. Tumor growth

C. MicroRNA levels

U87 cells → transfect with miR-378 exp. Vector → tumor xenograft model
microRNAs regulate tumor angiogenesis

• Pro-angiogenic microRNAs
  – miR-17-92 cluster: TSP-1, CTGF
  – miR-378: Sufu (suppressor of fused)
  – Let-7f

• Anti-angiogenic microRNAs
  – miR-221 and miR-222: c-Kit and eNOS
  – miR-15 and miR-16: VEGF and Bcl-2
  – miR-20a and -20b: VEGF and Bcl-2

More and more microRNAs are have shown to involved in tumor initiation, progression and maintainance
Tumor metastasis

Metastasis is the process by which a tumor cell leaves the primary tumor, travels to a distant site via the circulatory system, and establishes a secondary tumor.
miR-373 and miR-520c promote tumor metastasis in vivo

11/16 mice developed metastasis
9/15 mice developed metastasis
0/10 mice developed metastasis
microRNAs involved in all aspects of cancer features
Mechanisms that link microRNA to disease

- **Genomic alterations of microRNA**
  - Chromosome deletion, amplification, and translocation
  - Single nucleotide polymorphism of miRNA or miRNA targets

- **Alteration on the expression levels of miRNA**
  - Transcriptional control: transcription factor, enhancer, repressor
  - Epigenetic modification: DNA methylation, histone acetylation

- **Alteration on the processes of microRNA biogenesis**
Genomic Alterations

Change in miRNA expression levels

Change in miRNA target spectrum

Effect on target

Rearrangements

Strong promoter
Weak promoter

SNP

Effect on target
Transcriptional Regulation

Transcription factors: c-myc, Ap1 (c-Jun/c-Fos), p53, NFkB, HIF-1

DNA methylation: DNMT1, DNMT3A and DNMT3B

Histone modifications: acetylation, methylation and phosphorylation
Myc regulates the expression of miR-17-92 cluster
Epigenetic Gene Regulation

- **DNA methylation**
  - Promoter CpG islands and intergenic region

- **Histone post-translational modifications**
  - Acetylation, methylation, phosphorylation, ubiquitination, ADP-ribosylation

- **Chromatin remodeling**
  - ATP-dependent remodeling factors
Regulation of miRNA expression by promoter methylation

Expression of miRNA also regulated by other epigenetic mechanisms

[Cell Cycle 6:12, 1455-1459, 15 June 2007]
microRNA in Other Diseases
microRNAs regulate embryonic stem cells
miRNAs regulate innate and adaptive immune responses

Adaptive immune

Innate immune

Nature Immunology (2009) 9:839-845
Effects of virus-encoded microRNAs on viral and cellular transcripts

**Viral**

- **Maintain latency**
- **Cancer development**

**Cellular**

- Inhibits the host interferon response
- Contributes to pathogenesis and tumorigenesis in host
- Inactivate NK cells for immunoevasion

**HOST**

- EBV
- CXCL-11/I-TAC
- KSHV
- miR-K12-1
- miR-K12-3-3p
- miR-K12-6-3p
- miR-K12-11
- miR-BART1-5p
- miR-BART16
- miR-BART17
- miR-BHFR1-3
- THBS1
- ICP0/ICP4
- miR-H2-3p
- miR-H6
- ICP34.5
- miR-I
- IE1
- miR-UL112-1

**HOST**

- HCMV

Role of microRNA in heart development and function