Your Genes and Your Health

http://bio84.stanford.edu/

miRNA Regulatory Networks

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Announcements

• For those of you taking the course for Credit or for a Letter Grade:
  - Your 1-2 page paper for credit or a 4-5 page paper for a letter grade will be due March 16 by 7:00 PM (the time of last class).
  - Possible homework topics are on the course web site http://bio84.stanford.edu/.
  - You must send me your paper as an email or email attachment to brutlag@stanford.edu.
  - I do not accept hardcopy “papers”.
Steps in Gene Expression

Replication

DNA → Transcription → RNA → Translation → Protein → Function → Symptoms or Phenotype
Gene Regulatory Mechanisms

• Transcriptional Mechanisms
  – Type of promoters & RNA polymerase
  – Control of Transcription
  – Transcription Factors and TFBS

• RNA processing
  – Capping
  – Splicing and Alternative Splicing
  – Poly-Adenylation

• Translational Mechanisms
  – Micro RNAs (miRNAs) inhibit translation
  – Silencer RNAs (siRNAs or RNAi) degrading mRNA

• Epigenetic Mechanisms
  – DNA methylation
  – Histone modifications
  – Chromatin remodeling
Mechanism of Translation Mediated by Ribosomes

Bacteria

Human

Belasco, Nature Reviews Molecular Cell Biology 11, 467-478 (July 2010)
Regulation of Translation by microRNAs

• MicroRNAs can inhibit translation of mRNAs and cause degradation of mRNAs
• Micro RNAs form an RNA-Induced Silencing Complex (RISC) that can both inhibit translation and degrade mRNA
• MicroRNA RISC complexes bind to the 3’ UTR regions of mRNAs and stop translation and induce mRNA degradation
microRNA Biogenesis

The process of microRNA biogenesis begins with the transcription of the miRNA gene by RNA polymerase II (Pol II). The primary transcript (pri-miRNA) is processed by the RNase III enzyme Drosha in the nucleus, resulting in a precursor miRNA (pre-miRNA). The pre-miRNA is then exported to the cytoplasm by Exportin-5. In the cytoplasm, the pre-miRNA is further processed by the enzyme Dicer in complex with TRBP/Loquacious, yielding a miRNA:mRNA* duplex. This duplex is then cleaved by Dicer/TRBP/Argonaute, leading to the production of mature miRNA that is bound to Argonaute.
Mechanisms of Translational Regulation by miRNP Complexes

siRNA mediated degradation of mRNA

versus

miRNA mediated inhibition of mRNA translation

Dicer Mechanism
(New York Museum of Modern Art - MOMA)
miRNA Expression Results in Temporal and Spatial Reciprocity with Target Expression
Predicted miRNA Precursors

Human miRNAs (March 9, 2016)

- Total number of miRNAs known: 28,645
- Number human miRNAs identified: 2,661
- Number of human mRNA targets: 34,788

- Each miRNA can have multiple mRNA targets (13 on average)
- Each mRNA can have multiple miRNA binding sites (each mRNA has 2 miRNA binding sites on average)

miRBase @ http://www.mirbase.org/
MicroCosm @ http://www.ebi.ac.uk/enright-srv/microcosm/ u.francke.hgjc.10/9/07
Homology Between C. elegans and Homo sapiens miRNAs

Thousands of microRNAs act in multiple biological events

- Developmental timing
- Differentiation
- Aging
- Apoptosis
- Metabolism
- Cancer
- ... Cell fate/differentiation, Cell cycle...
ALTERATIONS OF MICRORNAS ARE FOUND IN EVERY TYPE OF HUMAN CANCER

miRNA expression profiles classify human leukemias and carcinomas

Profiling miRNA expression using custom microarrays
miRNAs as Oncogenes and Tumor Suppressors
miRNAs Involved in Human Cancer

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Gene Loci</th>
<th>Cancer association</th>
<th>Function*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR15,</td>
<td>chromosome 13q14</td>
<td>Frequently deleted/downregulated in B-cell chronic lymphocytic leukemia. Negatively</td>
<td>TS</td>
<td>Cain, 2002</td>
</tr>
<tr>
<td>miR-16</td>
<td></td>
<td>regulates the antiapoptotic gene, BCL2.</td>
<td></td>
<td>Cimmino, 2005</td>
</tr>
<tr>
<td>miR-143,</td>
<td>chromosome 5q3233</td>
<td>Decreased abundance in colorectal cancer. Down-regulated in breast, prostate, cervical, and lymphoid cancer cell lines. miR-145 decreased in breast cancer.</td>
<td>TS</td>
<td>Michael, 2003</td>
</tr>
<tr>
<td>miR-145</td>
<td></td>
<td></td>
<td></td>
<td>Iorio, 2005</td>
</tr>
<tr>
<td>miR-21</td>
<td>chromosome 17q23.2</td>
<td>Antiapoptotic factor. Upregulated in glioblastomas and breast cancer.</td>
<td>OG</td>
<td>Chan, 2005</td>
</tr>
<tr>
<td>let-7</td>
<td>multiple loci</td>
<td>Negatively regulates the Ras oncogene. Directs cell proliferation, differentiation.</td>
<td>TS</td>
<td>Ciafre, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased abundance in lung cancer.</td>
<td></td>
<td>Iorio, 2005</td>
</tr>
<tr>
<td>miR-142</td>
<td>chromosome 17q22</td>
<td>t(8,17) translocation that places the MYC oncogene downstream of the mir-142 hairpin resulting in an aggressive B cell leukemia due to MYC over-expression.</td>
<td>N/A</td>
<td>Lagos-Quintana, 2002</td>
</tr>
<tr>
<td>BIC/miR-155</td>
<td>chromosome 21q21</td>
<td>Upregulated in pediatric Burkitt’s lymphomas, Hodgkins, primary mediastinal and diffuse large B cell lymphomas. Upregulated in human breast cancer.</td>
<td>OG</td>
<td>Eis, 2005</td>
</tr>
<tr>
<td>miR-17-19b</td>
<td>cluster chromosome 13q3132</td>
<td>Upregulated by the c-Myc oncogene. Negatively modulates E2F1 oncogene. Loss of heterozygosity of cluster in hepatocellular carcinoma. Over-expressed in B cell lymphomas.</td>
<td>TS/OG</td>
<td>He, 2005</td>
</tr>
</tbody>
</table>

*Abbreviations: TS, tumor-suppressor gene; OG, oncogene; N/A, not applicable
miRNAs and Cancer – A Summary

• miRNAs control cell cycle, cell differentiation and apoptosis by regulating oncogenes and tumor suppressor genes

• miRNAs are misexpressed in cancer and are therefore excellent diagnostic/prognostic markers in cancer

• Some miRNAs e.g. mir-155, can cause cancer and oncogenic miRNAs may be therapeutic targets in cancer

• Other miRNAs like let-7, may prevent cancer and may be therapeutic molecules themselves.

• MicroRNAs could augment current cancer therapies.
References

• Role of miRNAs in Cancer and Apoptosis
  Lynan-Lennon Biol Rev Camb Philos Soc. 2009 Feb;84(1):55-71
• Causes and consequences of microRNA dysregulation in cancer
• miRNAs as oncogenes and tumor suppressors. Zhang Dev Biol. 2007 Feb 1;302(1):1-1
• Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?
• Post-transcriptional gene silencing by siRNAs and miRNAs.
• A three-dimensional view of the molecular machinery of RNA interference.
• miRNAs and Cancer AAAI Science Webinars February 20 with George Calin, Brank Slack and Scott Hammond
• Raising the estimate of functional human sequence. Pheasant Genome Res. 2007 Sep;17(9):1245-53.