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HST.161 Molecular Biology and Genetics in Modern Medicine
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Lecture 23

RNAi

History, mechanisms and applications of RNAi

RNAi: a small collection of tools and endogenous mechanisms to use RNA to silence genes

- double-stranded RNA trigger for RNAi
 - o silencing pathway outlined on slides
 - o siRNA = short interfering RNA
 - o results in post-transcriptional, pretranslational cleavage, and gene is wiped out

siRNA has molecular hallmarks of RNaseIII-like cleavage

- important part of recognition is the overhanging segments of nucleotides

Broader definition of RNAi (more colloquial): includes miRNA

- microRNA (miRNA) are related but distinct from siRNA; come from endogenous genes, are single-stranded, but intersect with RNAi machinery
- miRNA and siRNA can both be incorporated into micro-ribonucleotide protein complex (miRNP)
- two pathways for gene silencing: co-translational silencing (miRNP binds imperfectly), or post-transcriptional, pre-translational cleavage (miRNP binds perfectly). Co-translational silencing **may** happen because cell recognizes this mRNA translation as being faulty, and so disperses it to p-bodies which destroy it.
- RISC (RNA-induced silencing complex) is not identical to miRNP

Multiple unique miRNAs may target the same mRNA; one miRNA can target multiple genes.

- Current research is looking at what miRNAs goes to what mRNA.
- Binding sites in mRNA tend to be in the 3' UTR; mutations and polymorphisms in these sites can lead to serious problems.
- There can be great diversity in the 3' UTR, since it's not constrained by a need to be very specific, like coding regions are. Finding homology across these regions is significant.

Biogenesis of miRNA

- See slide 9 for mechanisms
- hairpin region cuts out about 70 nucleotides

miRNA gene architecture

- transcribed from pol-2 promoters, like protein-encoding genes
- can be intergenic, or intragenic
 - o for intragenic miRNA, you either translate the whole protein normally, or you skip the exon that the miRNA is taken from
- actual miRNA creation looks much more complicated than these simple diagrams

Very oversimplified chart of siRNA and miRNA comparisons

- generally the way to think about siRNA is that they silence in “cis” – they target the gene itself, whereas miRNA silence in “trans”, from another location

The numbers of miRNAs we’re discovering seem to be plateauing, but it’s likely that there are still many, many small RNAs that aren’t exactly miRNAs but function similarly.

Other RNAi Mechanisms –

- likely derived from other eukaryotes, probably not mammals
- some small RNAs may work in the **nucleus** to silence, or activate, transcription
- RITS: RNA-induced transcriptional silencing complex; found in yeast, plants, and worms; **might** occur in mammals. Can bind to either DNA; can modify DNA itself or histones. May bind to RNA as well.
- siRNA amplification: amplifies siRNA to make silencing more robust. Single complex can cut multiple mRNAs (mammals). In worms and plants, enzyme called RNA-dependent RNA polymerase (RdRP – not present in mammals) uses siRNA to template-prime another strand of long dsRNA, creating second-generation siRNAs with different registers, and increased or decreased potency.

piRNAs: piwi-associated RNAs

- endogenous, ssRNA expressed only in germinal cells
- expressed from a few discrete loci and only from one strand; Dicer-independent, don’t form hairpin precursors
- expression is coupled to transposons and have sequences similar to transposons
- piRNAs likely involved in genome defense; however, their roles and mechanisms of action are still emerging.
 - o Ping-pong mechanisms: the way piRNAs work seems to be a hand-off between AGO3 and Piwi/Aub – see diagram on slide (from Cell, 2007)

RNAi as an experimental tool

- has revolutionized biomedical research
- identify novel gene **functions**, not just sequences
- microarray definition of whole genetic **pathways**
- RNAi genome projects
- Selectively removes individual gene expressions: compare to classical biochemical methods such as immunodepletion
- Generates hypomorphic states: permits more than one function to be ascribed to a particular gene
- Permits analysis of genes whose knockout is lethal
- Practical: rapid and less expensive than knockout methods

Principal advantages of chemically-synthesized siRNAs

- immediate onset of knockdown
- readily transfectable even in several primary cells
- easily titratable knockdown

- available from multiple vendors

Principle advantages of lentivirus-delivered siRNAs

- heritable/stable knockdown
- test gene function in primary cells and physiological conditions
- renewable resource
- available through RNAi Consortium

Potential therapeutic applications of RNAi

- miRNA expression profiling classifies cancers
- There are more than 500 miRNAs in humans possibly targeting more than 1/3 of human genes.
- Expression of many miRNAs change relative to untransformed cells
- In addition to being markers of cancer, do miRNAs cause cancers? Yes!
- miRNAs as markers: are they different from other markers? Yes, and they may be better than other markers. miRNAs have many targets. Altering certain combinations may have therapeutic effect.
- miRNAs that participate in cancer formation: are they oncogenes or tumor suppressors? How do they cause cancer?
- Are miRNAs targets for therapy?
 - o They may be druggable targets; cell delivers them to very specific sites. Most therapies under investigation use siRNAs to target mRNA.

In 9 years since the discovery of RNAi, there are already about 10 clinical trials to use these therapeutically.