Dr

RNAi

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What is RNAi?

- RNA-based mechanisms of gene silencing. These siRNAs are bound by a protein-RNA complex called the RNA-induced silencing complex (RISC)

Mello and Fire

- In 1998, researchers Craig C. Mello of University of Massachusetts Medical School and Andrew Z. Fire of Stanford University published ground breaking work regarding RNAi (Intro to Biotech, Pg. 50).
- In which they used double stranded pieces of RNA(dsRNA) to inhibit or silence expression of genes in the nematode roundworm (C. elegans).

Mello and Fire (cont'd)

- This naturally occurring mechanism for inhibiting gene expression is RNAi.

Nobel Prize 2006

- In 2006, Mello and Fire were rewarded the Nobel prize for their discovery of turning off genes.

How does it work?

- RNAi process engages the participation of several pathways.
- Two pathways are RNA introduction and mRNA degradation.
- In addition, an unclear amplication pathway exists.

How does it work? (cont'd)

- Insertion of double stranded or small interfering RNA into a cell can be accomplished in several ways.
- Bombardment, RNAi vector, and Cationic oligopeptides.

How does it work? (cont'd)

- Bombardment,agrobacterium,viral mediated dsRNA transfer or by infiltration.
- RNAi vector-to produce stable or transient dsRNA in vivo.
- In other words, the plant must be transformed with a vector that produces dsRNA, using the above techniques.

Unamalai (2004)

- Recently devised a way to introduce dsRNA without transforming the plant with RNAi vector.
- They use cationic oligopeptides for delivering dsRNA into plant cells.
- The scientists introduced dsRNA into tobacco cells using POA, which is a cationic oligopeptide 12-mer.

Unamalai (cont'd)

- Results show efficient silencing of the target genes.
- Path of mRNA degradation begins with a piece of dsRNA is introduced into the cytosol resulting in the recruitment of a dicer.
- This initiates chopping of long dsRNA into a number of small double-stranded fragments. (21-25 base pairs w/ 3' two nucleotide overhang)

Unamalai (cont'd)

- Attached w/ 5'and 3' ends, are siRNAs are incorporated into (RISC) via a undetermined pathway, initiated the process of mRNA degradation.
- A physical interaction occurs between dicer and (RISC) through a common PAZ domain.
- Thereafter, (RISC) utilizes the siRNA as a target sequence seeking the complementary mRNA.

Unamalai (cont'd)

- Successful docking of the RISC-siRNA complex at the targeted mRNA site initiates the degradation process.
- The mRNA degradation is completed by the action of the cellular exonuclease.

Third Step

- A third, but not yet fully understood, pathway seems to enhance effectiveness of RNAi, through the amplication of siRNA.
- Current consensus on the issue is that the siRNAs undergo amplication by an RdRP (RNA-dependent RNA polymerase).
- The site of siRNA amplication is yet to be determined.
- siRNAs might be involved in the synthesis of long dsRNA.

Secondary RNAs

- Further studies into RdRP mediated amplication revealed the presence of a secondary siRNA.
- Secondary RNAs, not detectable in the introduced dsRNAs are derived from siRNAs that complement the targeted mRNA.
- These secondary RNAs actively participate in the degradation of complementary mRNA.

utilize

Viruses

- Another realm to use RNAi is to cure viruses.
- Possible to add a RNAi sequence into an organism, resulting cells turning immune defenses on.
- Fire and Mello successfully tested these viral defense aspects on simple organisms like plant life and worms.
- More research is needed to see RNAi may help humans to fight cancer, viruses, and genetic defects.

Results

- To use bacteria, retro viruses, DNA viruses, lipidoids, and amphoteric liposome as vessels for RNAi.

Bacteria

- Diet of C. elegans in the lab is bacteria.
- Scientists engineer bacteria to make short bits of RNA needed for RNAi and feed it to the worms.
- The worms will incorporate the RNA from the bacteria into their cells.
- Method is not used in humans due to the immune system reaction of large amounts of foreign RNA.

Retroviruses

- Since viruses work by delivering genetic material into a cell, has been long viewed them as a potential method of delivering DNA or RNA into a cell.
- Lentiviruses, a family of retroviruses that includes HIV, use RNA as their genetic material used to deliver RNA to the cells of live mammals (including humans) for RNAi.
- The only ones to work w/ non-dividing cells

DNA viruses

- Adenoviruses and Herpes simplex-1 virus, both double-stranded DNA, studied as a RNAi vector.

Lipidoids

- Molecules that are chemically similar to fat molecules (lipids).
- Small RNA bits are chemically attached to lipidoids which carry them into the cells.
- Scientists engineer a “library” of over 1200 unique lipidoid molecules, test which ones to use for RNAi delivery.

Amphoteric Liposomes

- Particles, smaller than a cell.
- Made of a double layer of fat molecules.
- Both have acid and base properties.
- Current research in RNAi has called them “smarticles”.
- The unique properties, make it possible to precisely control the way they interact w/ the cells in the body, useful in RNAi.

Conclusion

- RNAi has many uses as to fight cancer, viruses, and genetic defects.
- My personal opinion within the next decade or so, we might see the cure for cancer or AIDS by using RNAi.