Lentiviruses: Slow and Steady wins the race
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Outline
- The “Slow” virus
- Turning the Tables, bad guy makes good
- Using a lentivirus to stop a lentivirus
- Making a lentivirus vector
- Evading detection
- Breaking the retrovirus “rules”

The Slow Virus
- Subclass of Retrovirus
- Replicates exclusively in nucleus
- Complex gene expression/regulation vs. simple retrovirus
- “lenti” Latin for SLOW
- Persistent infection, incubation could take years
- Lysogenic in differentiated cells
- Differentiated cell factors

LOOK FAMILIAR???
Bad guy virus makes good
Don’t judge a virus by it’s epidemic.
Phil Sharp Nobel Laureate
- Lentivirus vector induced siRNA inhibits HIV pol expression by selective binding and disruption of gag gene

Making a Lentivirus Vector I
- 3 Plasmid Co-Transfection
  1) Most trans-acting viral genes Plasmid
  2) VSV pseudotyped env gene Plasmid**
  3) YOUR FAVORITE GENE and cis-acting elements for packaging, RT, and integration Plasmid

Making a Lentivirus Vector II
- RECIPE REVIEW
  3 essential cis-acting elements
  - Packaging signal “E” TAKE ME!!!!
  - Reverse Transcription COPY ME!!!!
    - primer binding site
    - terminal repeat (R) guides RT from RNA -DNA
  - Purine rich 5’ of 3’ LTR region
  - LTR Integration sequences INTEGRATE ME!
    - Internal Promoters or TEs
CLAIM TO FAME: Gene Therapy

- Lentiviruses are the ONLY retroviruses able to integrate into chromosome of Non-Dividing Cells!!!
  - Matrix proteins hijack nuclear import machinery gets into nucleus
  - Uses cellular nuclear replication machinery
  - Up to 6 months sustained expression

Reducing the Risks

- Non-Replication-Competent Recombinant (non-RCR) vectors
  - Suppressed Plasmids for viral transcription
- Self-inactivating (SIN) vectors
  - LTR deleted to suppress transcriptional promoters
  - Does that mean you do posses DNA?

Can’t Catch Me!!

- Low antigen and viral expression
  - Incomplete integration
    - Requires factors from differentiated non-mitotic cells for integration
  - Lots of mRNA splice variants more than retroviruses
  - MULTISITE VARIATION
    - Restricted Expression pathways
- High env mutation
- Immune response increases severity of infection

Breaking the retrovirus “rules”

1) Active cell cycle required
   - Lentivirus gets into nucleus and replicates slowly in quiescent NON-MITOTIC differentiated cell
   - Lentiviral unique effectors for hijacking nuclear localization and replication machinery

2) Integrated viral DNA is the most efficient template for retroviral RNA transcription
   - Majority of Lentiviral provirus remains NOT integrated
   - Free ds linear DNA also does NOT induce cell

References