LENTIVIRAL VECTORS

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LENTIVIRAL VECTORS

- Human immunodeficiency virus (HIV) is a lentivirus that infects both dividing and non-dividing cells
- Use of the HIV virus as a viral vector has required the reengineering of the virus to achieve safe gene transfer
- Since HIV normally targets CD4 cells, replacing the HIV envelope gene with vesicular stomatitis virus glycoprotein (VSV-G) expands the infectious range of the vector and modes of transmission

LENTIVIRAL OCCUPATIONAL EXPOSURES

- Lentiviral (LV) risks in research settings primarily involve the inadvertent transduction of the lab worker
- These include the potential harmful effects of the transgene, insertional mutagenesis, or the activation of neighboring genes from vector integration or generation of replication competent lentivirus (RCL)

GENE THERAPY

- Gene therapy is a technique for correcting defective genes responsible for disease
- While genes could be repaired, swapped or up/down regulated, most current methods involve inserting normal genes into non-specific regions of the genome
- Targets genetic deficiencies (e.g., severe combined immunodeficiency syndrome -SCID) or cancer cells (e.g., advanced metastatic melanoma)

GENE THERAPY

- 5 children treated with retroviral vector containing IL2RG gene for X-linked SCID developed leukemia 3-6 years after treatment (insertional mutagenesis)
- Vector inserted into the chromosome near the LMO2 gene which has been implicated in several Acute Lymphoblastic Leukemia (ALL) translocations
- 1/10 gene therapy patients with Wiskott Aldrich syndrome (X-linked heme disorder) developed ALL
- None of the 20 adenosine deaminase (ADA) SCID patients developed ALL

RNAi

- RNAi was chanced upon when genetic engineers sought to insert the purple gene into a purple petunia to create a deeper purple flower
- This resulted in a white pigment-free flower which confounded the researchers
- This was subsequently discovered to be due to double stranded RNA (dsRNA) which is not normally found in human cells

SHORT INTERFERING RNA (siRNA)

- Cytoplasmic delivery of short interfering dsRNA (siRNA) is normally due to viral and other exogenous sources
- Human cells identify this as foreign and cleave it into siRNA or short 21-23 nucleotide long sequences by Dicer, a ribonuclease III enzyme
- These short duplexes are incorporated into a protein complex called the RNA-induced silencing complex (RISC)

siRNA

- RNA induced silencing complex (RISC) then unwinds and separates the dsRNA through the protein Argonaut 2 contained within the RISC complex
- The antisense single strand (or guide strand) targets complementary mRNA sequences where it binds and inactivates them shutting down protein synthesis
- When siRNA is delivered to the cytoplasm, the effect is relatively transient lasting up to 7 days in rapidly dividing cells and up to several weeks in resting cells
- This is why the purple gene was inactivated

ONCOGENES

- Tool in cancer research to modify cells to express oncogenes with transfer of modified cells back to animal
- Risk of accidental exposure with an oncogenic vector to laboratory staff
- Tumors that are the result of hazardous vector/transgenes will be marked by the integrated vector
- Structural information about the vectors (the DNA sequencing) needs to be maintained by the lab to determine whether a retroviral transduction is responsible for a future tumor

LENTIVIRAL OCCUPATIONAL EXPOSURES

- LV and retroviral vector exposures, particularly if associated with a hazardous transgene (e.g., an oncogene or toxin), should consider use of an antiretroviral agent
- These can include the reverse transcriptase and integrase inhibitors, but not the protease inhibitors

POST EXPOSURE PROPHYLAXIS FOR LENTIVIRAL EXPOSURES

- HIV transduction (reverse transcriptase (RT) and integration into the host genome) takes 12-24 hours
- Nucleoside and non-nucleoside reverse transcriptase along with integrase inhibitors blocked viral replication in lentiviral exposures even 12 hours post-exposure
- Murine leukemia viral (retroviral) vectors were inhibited by nucleoside but not non-nucleoside RT inhibitors
- Early treatment might reduce transduction of potentially hazardous vectors, but needs to be planned in advance and duration of treatment not established

RECOMMENDATIONS FOR HANDLING LENTIVIRAL AND RETROVIRAL VECTORS

- Use advanced lentiviral vector systems
- Avoid mixing systems
- Review potential for replication competent virus
- Avoid sharps and glass – anesthetizing animals
- Use PPE to avoid exposures to eyes, nose and mouth
- Containment within BSC’s when possible aerosol generation

RECOMMENDATIONS FOR HANDLING LENTIVIRAL AND RETROVIRAL VECTORS

- Consider risk for mutagenesis or toxic properties of transgene
- Consider risk from animals treated with LV
- Consider risk of viral shedding in immunodeficient animals
- Consider present or future risk for HIV in lab personnel along with confidential testing
- Maintain record of vectors especially post-accident
- Consider antiretroviral treatment post exposure

VECTOR/TRANSGENE HAZARDS

- Problems include what to monitor and for what length of time due to the potential for long latent periods
- Need to consider the consequences of exposure to the genetic insert when performing biosafety reviews and the additional issues with off-target effects or generation of replication competent virus
- Need to proactively train all staff to understand potential risks with these agents and on ways to prevent exposures
- Need to develop system to report and monitor exposures