Human Retrovirus Research Rotation Projects 2017

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1. **What HIV-1 Vpr Interactive proteins mediate G2 arrest?**
Background: Vpr induced G2 arrest is a key feature of HIV pathogenicity through binding DCAF1 ubiquitin ligase.
Methods:
1. Use Vpr-BioID (biotin ligase) fusion protein as well as mutants that fail to induce G2 arrest (Q65R, R80A) to transfect 293T cells to confirm biotin labeling of DCAF-1 (ubiquitin ligase substrate) and UNG2 (uracil glycosylase 2)
2. Submit biotin labeled proteins for mass spec analysis to identify proteins identified with wt but not mutant Vpr
3. Confirm mass spec identified proteins by coIP
4. Assess effect of siRNA against mass spec identified protein on ability of Vpr to induce G2 arrest
Ref: PMID: 28075409, 10864665

2. **What co-activators mediate IRF4 transcriptional activity in ATL**
Background: IRF4 is amplified or mutated (K59R) in 33% of HTLV-1 associated adult T cell leukemias (ATL) and over-expressed in all cases.
Methods:
1. Use IRF4-BioID (biotin ligase) fusion protein as well as mutant (K59R) to transfect Jurkat T cells and confirm interaction with BATF
2. Transfect IRF4-BioID and IRF4 (K59R)-BioID in ATL cell lines for mass spec analysis to identify interactive proteins
3. Confirm mass spec identified proteins by coIP
4. Assess effect of siRNA against mass spec identified proteins on ability of IRF4 to activate gene targets in ATL cells
Ref: PMID: 26437031, 27826752

3. **HTLV-1 Infection of Human Embryonic Stem Cells**
Background: HTLV-1 infects T cell precursor to produce clonal expansion of mature CD4+ T cells
Questions: At what stage of hematopoietic stem cell differentiation can HTLV-1 infect and what is the consequence?
1. Infect human induced pleuripotent stem cells with HTLV and assess hematopoietic progenitor numbers and clonality
Ref: PMID: 28408465

4. **What is the role of CTCF in HTLV-1 infection and pathogenesis?**
Background: HTLV-1 has a single binding site for CTCF, which is a chromatin barrier element
Methods:
1. Examine replication of CTCF-binding site mutant in vitro and in humanized mice
2. Examine lymphocyte immortalization by CTCF binding site mutant in vitro and in humanized mice
Ref: PMID: 26929370

5. How does HTLV-1 Tax activate the alternative NFkB pathway
Background: HTLV-1 replication of alt NFkB is important to confer resistance to apoptosis
Methods:
1. Make BioID (biotin ligase) fusions with wt and Tax mutant (deficient in alt NFkB activation)
2. Transfect Jurkat T cells to examine protein expression and alt NFkB activation (by p100 cleavage)
3. Biotin label transfected Jurkat cells to determine if known interactors bind Tax-BioID e.g. p100, NEMO
4. Perform mass spec analysis to identify interactive proteins
Ref: PMID: 24060211, 16751281

6. What cellular proteins regulate HTLV-1 entry into human cells
Background: Although Glut1 and Nrp1 have been proposed as HTLV-1 receptors, coreceptor and entry mechanisms remain obscure
Methods:
1. Screen Crispr/Cas9 knockout library of haploid cells for HTLV-1 entry using a luciferase virus
2. Compare results with prior genetic screen of genes that promote HTLV-1 entry into mouse cells
Ref: PMID: 21114861

7. What is critical coreceptor domain for HIV-1 entry
Background: HIV-1 env sequences in trimer interaction and gp120-gp41 interaction domains as critical for CXCR4 use
Methods:
1. Construct and test HIV-1 X4 env with residues from gp120-gp41 interaction and/or trimer interaction domain shown to be critical for CCR5 vs CXCR4 use and determine tropism with luciferase reporter viruses
Ref: PMID: 27128349