DEPARTMENTAL SEMINAR SERIES

Molecular Genetics
UNIVERSITY OF TORONTO

Temerty Medicine

HIV-1 assembly, maturation, and drug resistance

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The Freed lab's research is focused on various aspects of HIV-1 particle assembly, budding, envelope glycoprotein (Env) trafficking and incorporation into virus particles, particle maturation and post-entry events. HIV-1 drug resistance is also a long-standing interest. In the seminar on Nov. 6, 2023, two projects will be discussed in detail: the requirement for neutral sphingomyelinase 2 (nSMase2) in HIV-1 maturation, and the basis for resistance to integrase strand-transfer inhibitors (INSTIs). These projects are described below.

HIV-1 assembly occurs at the inner leaflet of the plasma membrane in highly ordered membrane microdomains. The size and stability of membrane microdomains is regulated by activity of nSMase2. To investigate the role of nSMase2 in HIV-1 replication, we either knocked down nSMase2 with siRNA or treated virus-producing cells with the nSMase2 inhibitor PDDC and monitored virus assembly, release, maturation, and infectivity. We found that disruption of nSMase2 impairs HIV-1 Gag and GagPol processing, resulting in a profound impairment in particle maturation and infectivity. EM analysis shows that disrupting nSMase2 in virus-producing cells alters the morphology of HIV-1 particles and disrupts the formation of the immature Gag lattice. These studies demonstrate a previously undescribed role for nSMase2 in the morphogenesis and maturation of primate lentivirus and suggest that specific alterations in the lipid composition of assembling virus particles profoundly impact late stages of the lentiviral replication cycle.

Some INSTI-treated individuals experience virological failure in the absence of resistance mutations in IN. To elucidate INSTI resistance mechanisms and pathways, we performed long-term passaging of lab-adapted and primary viral isolates using human T-cell lines or primary PBMCs over nearly one year with an escalating concentration of the INSTI dolutegravir (DTG). HIV-1 became resistant to DTG by sequentially acquiring mutations in Env and Gag-nucleocapsid (NC) in the absence of resistance mutations in IN. The NC mutations selected with DTG conferred modest (3-5- fold) resistance to INSTIs but not to an RT inhibitor. The DTG-resistant Env mutants exhibit resistance to multiple classes of ARVs, with the fold resistance being ~2-logs higher for INSTIs than for other classes of drugs. Viral transmission of these mutants through cell-cell contact is more efficient than that of WT, resulting in a higher multiplicity of infection (MOI) and reduced sensitivity to DTG. Overall, these findings demonstrate that, in cell culture, a combination of mutations in Env and NC can confer high-level resistance to INSTIs in the absence of IN mutations.

Host: Dr. Alan Cochrane

Date: Monday, November 6th, 2023
Time: 3:00 PM
Place: Room 254 (MC) Mechanical Engineering Bldg.