

POSTER 2**FIV ENV MUTATIONS ENHANCE CELL-CELL TRANSMISSION TO ESCAPE TSG-5'-MEDIATED INHIBITION OF BOTH FIV RELEASE AND CELL-FREE FIV INFECTION**

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We have shown that feline immunodeficiency virus (FIV) relies on a PSAP motif in Gag for efficient virus release in both human (HeLa) and feline (CrFK) cells via a direct interaction with Tsg101. Consequently, FIV release is inhibited by transient expression of TSG-5', a fragment of Tsg101 containing the PT/SAP-binding domain. To explore potential fates of targeting late domain function as an antiretroviral strategy, we created a CrFK cell line that stably expresses high levels of TSG-5'. CrFK/TSG-5' cells inhibit FIV and HIV-1 budding and FIV replication, thus providing the first example of a late-domain-specific inhibitor that constitutively blocks retroviral replication. To explore potential mechanisms of escape, we selected for FIV variants that replicate efficiently in CrFK/TSG-5' cells. Surprisingly, all resistance-conferring mutations mapped to the Env glycoprotein; specifically, the V3 loop of SU and the distal heptad repeat (HR2) of TM. These domains play critical roles in Env-mediated membrane fusion through interactions with heparan sulfate proteoglycans (HSPGs) and CXCR4. By electron microscopy, CrFK/TSG-5' cells infected with TSG-5'-resistant FIV mutants show long, extracellular filaments coated with mature virions. These filaments were only ~10 nm in diameter but extended several microns in length and may be part of an extracellular matrix. By an unknown mechanism, CrFK/TSG-5' cells are also highly non-permissive to cell-free infection, relative to control cells; this block can be bypassed by VSV-G pseudotyping. We have observed that the medium of cultured CrFK/TSG-5' cells becomes partially gelatinous at high cell densities, a phenomenon not seen in control CrFK cells. This TSG-5'-specific accumulation of viscous material may interfere with FIV infection. Consistent with this model, the supernatant alone from cultured CrFK/TSG-5' cells is sufficient to inhibit cell-free FIV infection of TSG-5'-negative CrFK cells. Using newly developed assays to quantify cell-cell transmission of FIV, we also show that Env mutations significantly enhance cell-cell FIV transmission, even in the absence of TSG-5', when diffusion of cell-free virus is physically limited by a semi-solid matrix (agarose). Thus, mutations in FIV Env appear to enhance cell-cell transmission to overcome TSG-5'-mediated restrictions at the level of both virus release and cell-free infection.