

## FIV RESISTANCE TO A DOMINANT-NEGATIVE FRAGMENT OF Tsg101 MAPS TO THE Env GLYCOPROTEIN

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Feline immunodeficiency virus (FIV) is known to cause AIDS in its natural host, the domestic cat, and is thus an important model for the development of new vaccine and antiretroviral strategies. Many HIV-1 components have already been targeted for drug therapy including reverse transcriptase, integrase, protease, and envelope. However, the potential for inhibiting the assembly and release of infectious virus particles has yet to be fully exploited. In this regard, we and others have shown that components of the Endosomal Sorting Complexes Required for Transport (ESCRT-I, II, and III) and ESCRT-associated factors are required for retroviral release and bind directly to one or more “late domains” found in Gag. Similar to HIV-1, FIV Gag utilizes a PSAP late domain to bind the ubiquitin E2-like variant (UEV) domain of the ESCRT-I component Tsg101. Overexpression of the N-terminal UEV domain of Tsg101 (TSG-5') specifically inhibits the release of HIV-1 and FIV. To explore the possibility of targeting late domain function as an antiretroviral strategy, we engineered feline CrFK cells to constitutively express high levels of TSG-5', providing the first description of a late-domain-specific inhibitor of retroviral replication that can be stably expressed. To further explore possible mechanisms of escape from this novel form of inhibition, we have selected for FIV variants that replicate efficiently in CrFK/TSG-5' cells. Surprisingly, we observed that the resistance-conferring mutations mapped to Env, in domains critical for membrane fusion. These Env mutations confer escape from TSG-5' without rescuing the TSG-5'-imposed inhibition of virus release. Intriguingly, EM analysis of FIV-infected CrFK cells shows numerous long, thin extensions from the plasma membrane that are highly coated with mature virions, suggesting a role for these structures in cell-cell transmission. By immunofluorescence microscopy, we have also observed the propensity for CrFK cells to establish highly extended, actin-based filopodial bridges between cells. Gag is observed to localize to these structures. Similar filopodial structures in cells producing MLV or HIV-1 have been described as “cytonemes” or “nanotubes”. We are currently exploring the hypothesis that these Env mutations provide escape from TSG-5'-mediated inhibition by stimulating the transmission of virus along extracellular cytonemes.