

Molecular Biology of Retroviruses

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Our research involves the molecular characterization of retroviruses and the development of ways to interfere with the retroviral life cycle. In particular, we use feline immunodeficiency virus (FIV) for the study of lentivirus infections. FIV causes an AIDS-like syndrome in domestic cats and has structural and functional similarities to HIV, the cause of AIDS in humans. Thus, developing ways to interfere with FIV infection may result in useful treatments for infections in both cats and humans. Our primary interests continue to be the molecular characterization of receptor interactions and the molecular basis for the development of drug resistance in the critical aspartic protease encoded as part of the enzyme cassette of all retroviruses.

RECEPTOR STUDIES

Like acute strains of HIV, FIV uses the chemokine receptor CXCR4 to enter the target cell, the CD4+ T lymphocyte. However, both HIV and FIV have other primary binding receptors that bind the virus as a prelude to interaction with the entry receptor. We think that these other receptors increase the effective local concentration of the incoming virus and alter the conformation of the surface glycoprotein to increase the binding affinity of CXCR4. Whereas HIV uses the cell-surface protein CD4 as a primary binding receptor, FIV uses the activation antigen CD134. CD134 is expressed on activated CD4+ T cells, a finding that explains why FIV can infect and kill CD4+ T cells, even though the virus does not bind CD4.

We have shown that interaction of the FIV surface glycoprotein gp95 with CD134 causes a conformational change in gp95. This conformational change increases the affinity of gp95 for CXCR4; similar changes occur when HIV gp120 binds CD4. Thus, there is an evolutionary conservation of the mechanism of infection by FIV and HIV, even though these 2 lentiviruses use different binding receptors. Results to date in both lentivirus systems support the notion that this mechanism protects certain epitopes on the surface glycoprotein from immune surveillance until the moment of virus binding and entry into the cell. We used a panel of neutralizing monoclonal antibodies to map the region of gp95 in which these CD134-dependent neutralizing epitopes reside.

In more recent studies, we used synthetic peptides containing the antibody-reactive region to map the epitopes recognized by the neutralizing antibodies. A cluster of these epitopes resides in the variable loop 3 of FIV gp95, consistent with the notion that this region is central to CD134 receptor binding. We previously mapped regions of feline CD134 receptor involved in interaction with gp95 by using chimeric proteins consisting of feline and human CD134 (the human homolog does not bind FIV glycoprotein) and site-directed mutagenesis. During the past year, we did similar mapping studies on gp95

to further define regions critical to CD134 and CXCR4 binding. So far, the results are consistent with the idea that variable loop 3 is the contact region for binding to both receptors. Cocrystallization studies are in progress to determine the structure of the region surrounding the antibody-binding sites. We are also using these antibodies to develop specific agents that interfere with receptor binding and may be useful as therapeutic agents.

PROTEASE DRUG RESISTANCE

The aspartic protease of lentiviruses is responsible for processing the viral Gag and Pol polyproteins that must occur at the proper time and in the proper sequence in order to generate infectious virus. Drugs against the HIV protease are key components of highly active antiretroviral therapy, a treatment regimen used successfully to treat, but not cure, patients infected with HIV. Both FIV and HIV encode an aspartic protease and although the FIV and HIV proteases are structurally similar, the 2 enzymes have unique sequence-cleavage properties. We have used the parallels and differences between FIV and HIV proteases to better understand the molecular determinants that govern substrate/inhibitor selectivity. We hope that our results will define the limits of plasticity of the 2 enzymes and lead to insights into the development of drug resistance.

As reported previously, we showed that the number of amino acid residues involved in the sensitivity of the proteases to drugs is limited, and we can markedly change the sensitivity of the FIV protease to be more like that of the HIV protease by changing as few as 4 amino acids around the active site. However, changing the substrate-cleavage specificity requires substantially more changes. These findings explain how the virus, when an infection is treated with a drug, can mutate to avoid the drug but retain sufficient substrate-cleavage specificity to allow proper Gag/Pol processing and generation of infectious virus. Critical to this process is maintaining the proper order of site cleavage in Gag/Pol, and changes in this order result in generation of noninfectious virus. We think that altering the order of cleavage, in addition to blocking protease activity, may be useful as a novel intervention strategy.

PUBLICATIONS

Heaslet, H., Lin, Y.C., Tam, K., Torbett, B.E., Elder, J.H., Stout, C.D. Crystal structure of an FIV/HIV chimeric protease complexed with the broad-based inhibitor, TL-3. *Retrovirology* 4:1, 2007.

Lin, Y.C., Brik, A., de Parseval, A., Tam, K., Torbett, B.E., Wong, C.H., Elder, J.H. Altered Gag polyprotein cleavage specificity of feline immunodeficiency virus/human immunodeficiency virus mutant proteases as demonstrated in a cell-based expression system. *J. Virol.* 80:7832, 2006.

Manuell, A.L., Beligni, M.V., Elder, J.H., Siefker, D.T., Tran, M., Weber, A., McDonald, T.L., Mayfield, S.P. Robust expression of a bioactive mammalian protein in *Chlamydomonas* chloroplast. *Plant Biotechnol. J.*, 5:402, 2007.

Whiting, M., Tripp, J.C., Lin, Y.C., Lindstrom, W., Olson, A.J., Elder, J.H., Sharpless, K.B., Fokin, V.V. Rapid discovery and structure-activity profiling of novel inhibitors of human immunodeficiency virus type 1 protease enabled by the copper(I)-catalyzed synthesis of 1,2,3-triazoles and their further functionalization. *J. Med. Chem.* 49:7697, 2006.

Index terms

AIDS. See HIV infection.

Feline immunodeficiency virus

as AIDS model

proteases of

receptors for

HIV infection

Receptors

for FIV

Viruses

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HIV

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