Chemical Library Screens Targeting an HIV-1 Nef/Host Cell Kinase Complex Identify Novel Anti-retroviral Compounds.

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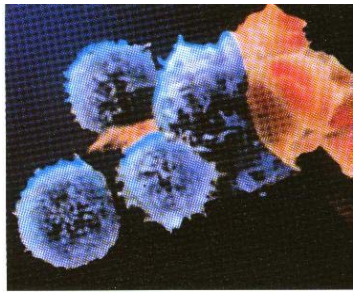
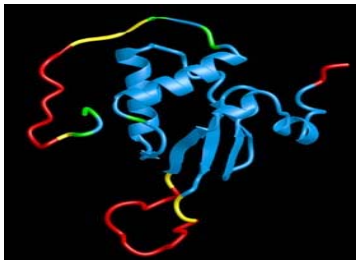
HIV-AIDS

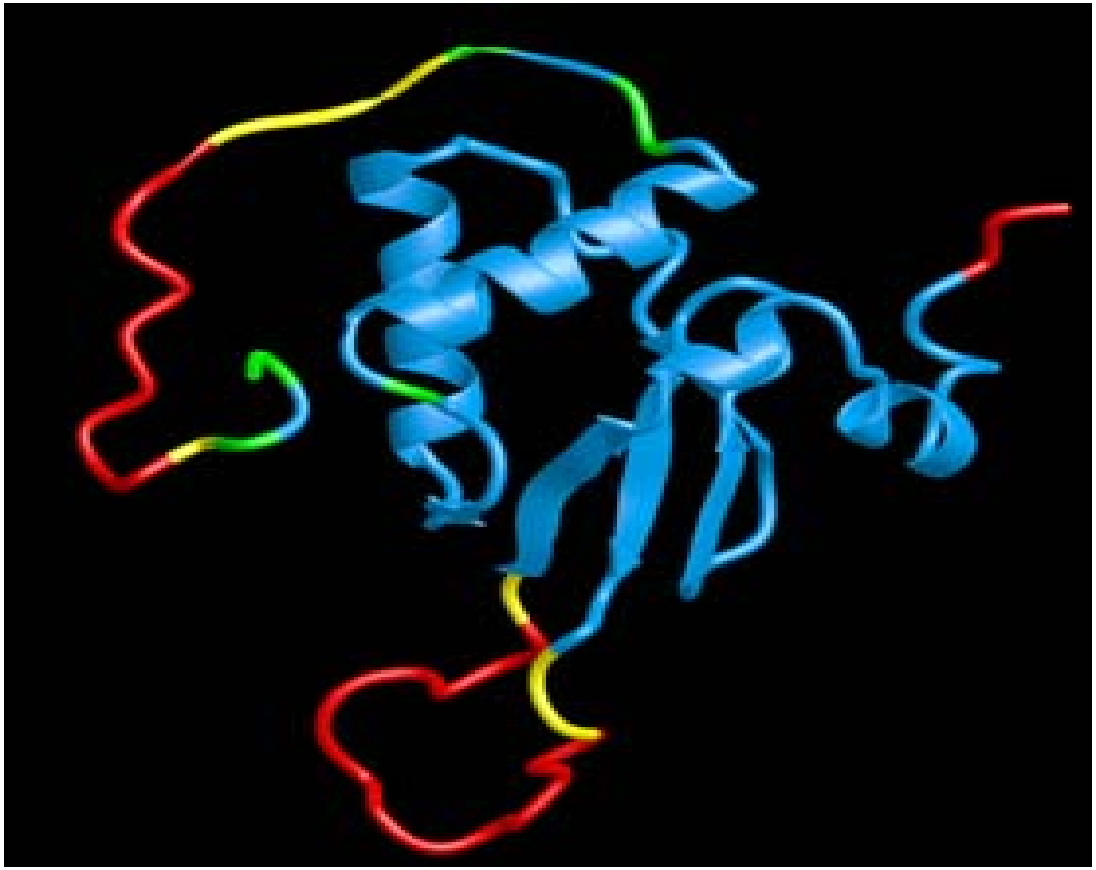


- HIV- Human Immunodeficiency Virus
- AIDS- Acquired Immune Deficiency Syndrome (NOW A PANDEMIC)
- Mechanism – T-cell, Macrophages, Dendritic cells
- 33.2 million in 2007
- 2.1 million (330,000)
- US Center for Disease Control and Prevention

Accessory Protein

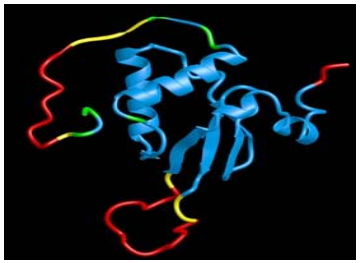


- HIV-1 encodes essential accessory proteins. **(Nef)** –
- Participants in the pathogenesis of AIDS, makes a good target for anti-HIV drug discovery.
- Interacts with **Hck** (Src family kinase) – regulating signal transduction
- These Nef mediated interaction optimize viral replication and contribute to the immune evasion as well as survival of infected cells.



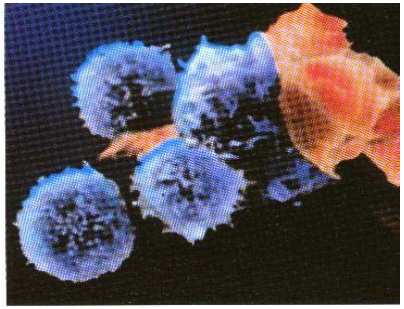
High-Throughput Screening Assay

- Inhibitors of Nef in a complex with one of its host cell binding partners.
- 10,000 discrete chemical compounds, unique **DIPHENYLFUROPYRIMIDINE.**
- Identifying the diphenylfuropyrimidine substructure as a valuable probe of HIV Nef function as a potential pharmacophore for future AIDS drug development.



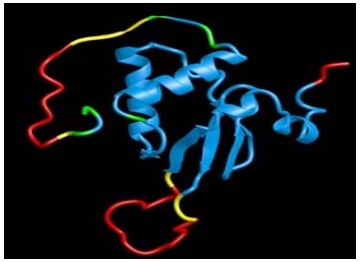
PURPOSE!!

- Inhibiting the function of the Nef protein and other HIV accessory factors and their interaction with host cell target proteins may accelerate the discovery of new anti-HIV agents.



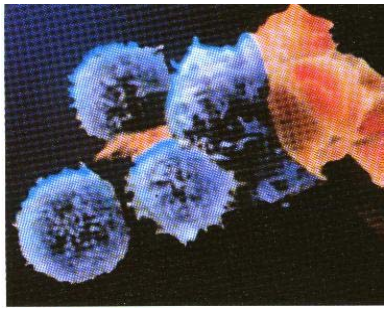
MECHANISM

- Nef binds to the Src 3 homology 3 (SH3) domains of the Src family members (Fyn, Hck, Lck, Kyn, and c-Src.
- Displacement of the SH3 domain from a negative regulatory interaction with the catalytic domain.
- Growing evidence shows that Nef:SFK interaction is an important interaction for HIV replication and AIDS progression.



APPROACH.

- Lack of catalytic function
- Couples Nef to the activation of Hck.
- These compounds represent valuable chemical probes for Nef-dependent HIV-1 replication in vitro.



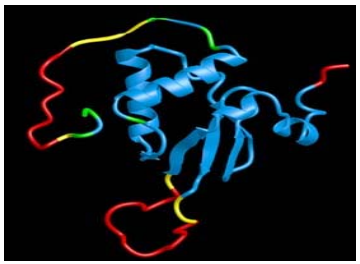
TESTING …

Test # 1:Suppression of Hck.

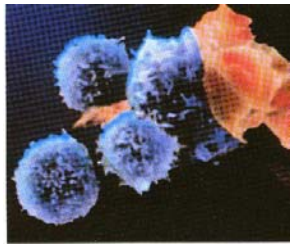
- Suppression of Hck protein level ------- HIV replication was blocked. (Komuro et al.)

Test # 2: Expressing Nef

- Transgenic mice, Expression of Nef to T-cells and macrophages induced an AIDS-like syndrome characterized by t-cell depletion, diarrhea, wasting and 100% mortality.



TESTING …



Test # 3: Depressing Nef

- Mice expressing a Nef mutant essential for SH3 binding showed no evidence of the AIDS-like phenotype.

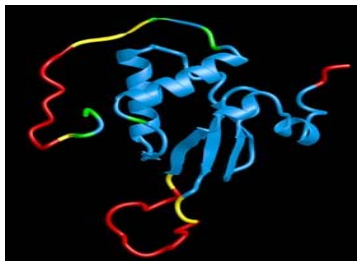
TEST # 4: Hck-null background

- Mice expressing wild-type Nef were crossed into a Hck-null background, appearance of the AIDS-like phenotype was delayed and mortality was reduced.

….CRITICAL ROLE OF Nef…

TESTING …

- SFK's adopt inactive conformation in vivo as a result of phosphorylation of a conserved tyrosine residue in their C-terminal tails due to a kinase Csk.
- Expressed and purified a form of Hck (Hck-YEEL)with a modified C-terminal tail that drives Hck down regulation independently of Csk.
- Mass spectrometry revealed the presence of a single phosphotyrosine residue in the C-terminal tail, indicating that the kinase was in the down regulation conformation.
- Nef:Hck kinase complex phosphorylates a single tyrosine residue in a synthetic FRET-peptide substrate that is tagged on the N-terminus with coumarin and on the C-terminus with flourescein.
- The reaction is then developed with a site specific protease that selectively cleaves the non-phosphorylated FRET-peptide.
- The uncleaved phosphopeptide maintains the FRET signal.



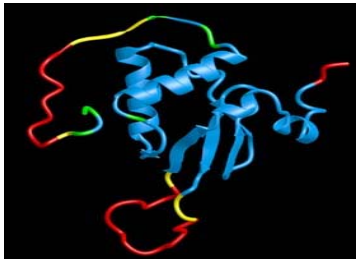
TESTING …HcK-YEEL activation dependent on the presence of Nef???

- FRET-peptide substrate phosphorylation increased as a function of the amount of the Hck-YEEL added to the assay. Kinase activity was likely due to random intermolecular collision of the Hck-YEEL
- This experiment was then repeated in the presence of a 10-fold molar excess of HIV-1 Nef at each Hck-YEEL concentration. The presence of Nef markedly shifted the Hck-YEEL activation curve to the left, indicative of its ability to bind to Hck and inhibit kinase activity.

……Keep in mind when Hck is phosphorylated it is inactive so when Nef inhibits kinase activity the Hck is activated.

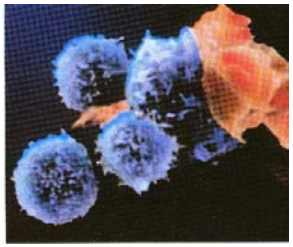
IDENTIFICATION OF Nef:Hck INHIBITORS BY HTS.

- The Nef:Hck-YEEL complex was then used to screen chemical libraries consisting of approximately 10,000 discrete compounds for inhibitory activities.
- The libraries of chemicals were populated with structures biased towards kinase and phosphate inhibitors.
- Results gave 3 chemicals. All three confirmed inhibitors were obtained from kinase inhibitor-biased library.



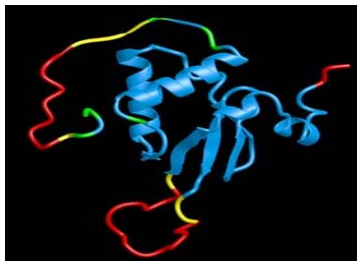
Inhibitors..

- They used **U87MG astroglioma cells** that were engineered to express HIV-1 receptors CD4 and CXCR4. In these cells HIV replication is dependent upon Nef.
- These cells were infected with HIV-1 as well as an isogenic variant that fails to express Nef. RESULTS show that the wild-type HIV replicated 60-folds more efficiently that the Nef mutant in these cells.
- U87MG cells were infected with wild-type HIV-1 in the presence of each compound at 5μM and viral replication was assessed. RESULTS: All 3 compounds showed anti-HIV activity but compound 3 was most remarkable, suppressing HIV replication to undetectable levels in this experiment.



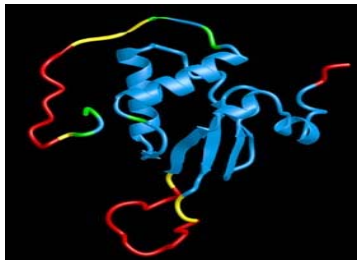
Inhibiting..

- They concluded after testing different analogs of the chemical compounds, that 4-amino substituted DFP block HIV through a Nef-dependent mechanism. And none of these compounds displayed cytotoxicity.



In conclusion

- Majority of current drugs used to treat HIV target and inhibit the proteins involved in the replication of the virus such as reverse transcriptase or interfere with virus host cell fusion.
- Work in this report support the concept that Nef is an important target for anti-HIV drug discovery.



ACKNOWLEDGEMENTS

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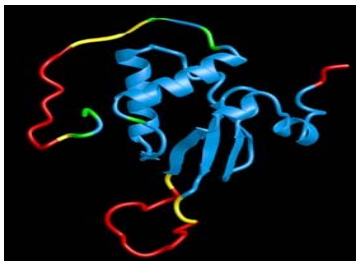

ANY QUESTIONS??