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Research Progress of HIV-1 Nef Inhibitors

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Abstract

HIV-1 infection poses a major threat to the public health worldwide. The antiretroviral agents that are currently used to treat HIV-1 infection target viral reverse transcriptase, integrase and protease, or block the fusion of viral envelop and cell membrane. Studies have shown that the HIV-1 encoded protein Nef plays an important role in the pathogenesis of viral infection. Nef ensures efficient counterattack against host immune responses as well as long-term evasion of immune surveillance. In addition, Nef, expressing at a high level early in the viral life cycle, is required for maintaining a high viral load in the persistent infection in vivo and for full pathologic potential. Therefore, Nef may be an excellent target to treat HIV-1 infection. In this manuscript, we reviewed five potential Nef inhibitors, namely, DLC27-14, tightly bound hydroxypyrazole HIV-1 Nef inhibitor B9, 2c-like inhibitors, N-(3-aminoquinoxalin-2-yl)-4-chlorobenzenesulfonamide and compound 1[(7-oxo-7H-benzo[anthracene]-3-yl)amino]anthraquinone, and their working mechanisms. These drugs may be further developed into new regimens for the treatment of HIV-1 infection. (AIDS Rev. 2020;22:221-226)

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Key words

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Introduction

Human immunodeficiency virus (HIV)-1 infection poses a major health threat to the public. Without treatment, it can cause acquired immune deficiency syndrome. The antiretroviral agents that are currently used to treat HIV-1 infection mainly target the viral reverse transcriptase, integrase and protease, or block the fusion of viral envelop and host cell membrane[1,2]. As one of the HIV-1 accessory proteins and the master regulator of various host cell processes[3], Nef plays an important role in viral replication *in vivo*[4]. Nef is expressed at a high level in infected cells soon after viral entry and needs to be attached to cell membrane to function[5,6]. Deletion of *nef* from viral genome impairs viral replication and delays disease progression[7]. Down-regulation of the expression of CD4, MHC-I, MHC-II, and CD28 molecules from the surface of HIV-1-infected cells is among Nef's most prominent functions studied so far. In consideration of Nef's critical role in HIV pathogenesis, Nef inhibitors have been explored as a potential category of anti-HIV drugs in recent years. These agents can not only interfere with HIV-1 life cycle but also enhance the recognition of viral-infected cells by the host immune system as part of the strategy to clear HIV-infected cells. In this manuscript, we reviewed five types of Nef inhibitors that are currently under development and their working mechanisms, which are illustrated in figure 1.

DLC27-14, an improved Nef inhibitor acting on SH3-Hck complex

The cellular proteins interacting with Nef can be divided into two groups: proteins involved in the trafficking of cell surface receptors and those involved in cell signaling pathways[8]. Most of these protein-protein interactions are mediated through Nef core domain, a stable globular domain containing a proline-rich motif that allows the binding of Src homology 3 (SH3) kinase domain[9]. The biological property and the availability of the structural data of Nef-SH3 domain complex make Nef a suitable target for structure-based drug development. DLC27, the first-generation of Nef inhibitor, inhibits – Nef-SH3domain interaction by specifically blocking the region of Nef that is required for its binding to the arginine-threonine (RT) loop of SH3 domain. The RT loop binding region in Nef consists of a mostly hydrophobic groove delimited by residue Trp113, and residue Arg96[7,10] in the groove is essential for Nef:SH3 interaction.

DLC27-14, the second-generation of Nef inhibitor, acts as a special protein disorder catalyst that destabilizes the folded Nef core domain. It is an improved inhibitor of Nef:SH3 complex. The only compatible docking mode of action for DLC27-14 suggests that it promotes the opening of two α-helices of Nef and destabilizes the core domain, thereby increasing the sensitivity of Nef to HIV protease and subsequent proteolysis. Therefore, from one point of view, DLC27-14 acts as a more effective inhibitor of Nef:SH3 complex formation than DLC27.

B9: targeting the dimeric helical area of HIV-1 Nef

An unique inhibitor that is based on hydroxypyrazole scaffold and named B9 was found through a high-throughput screening with a high level of specificity (IC_{50} 2.8 mm) in blocking Nef's such activities[11] as enhancement of viral infectivity and MHC-I downregulation[12]. The chemical denomination of B9 is (E)-4-((3-chlorophenyl) diazenyl)-5-hydroxy-3-nitro-1H-pyrazole-1-carbothioamide. It binds to the active site of Nef that is located at the helical dimeric region in the helix dimer zone. Therefore, targeting this area inhibits Nef dimerization.

B9 has a three-ringed structure with a diazene linker connecting a pyrazole core to a chlorophenyl group. However, B9 also has several structural defects such as an azo linker that leads to low oral bioavailability. Thus, to improve its oral bioavailability, the azo linker in B9 was replaced with a one or two carbon linker. The obtained non-azo analogs retained their nanomolar binding affinity to Nef and inhibited HIV-1 replication at the micromolar potency without significant cytotoxicity in cell-based assays. Computational docking studies showed that these non-azo analogs occupied the same predicted binding site as the original azo compounds at the dimeric interface of HIV-1 Nef. Pharmacokinetic evaluation of the non-azo B9 analogs in mice showed that replacement of the azo linkage significantly increased their oral bioavailability without substantially affecting their plasma half-lives or clearance. JZ-96-21 and JZ-97-21, namely, the non-azo derivatives of B9, are orally bioavailable with reduced cytotoxicity. The improved oral bioavailability of these non-azo derivatives provides a starting point for further drug optimization in support of future efficacy testing against Nef activities in animal models[13].

Based on the structural and pharmacophoric features of B9, a potential Nef antagonist was recently identified[14]. The compound libraries (shape similarity- and pharmacophore-based ligand libraries) were first generated based on a hybrid virtual screening. Then, the

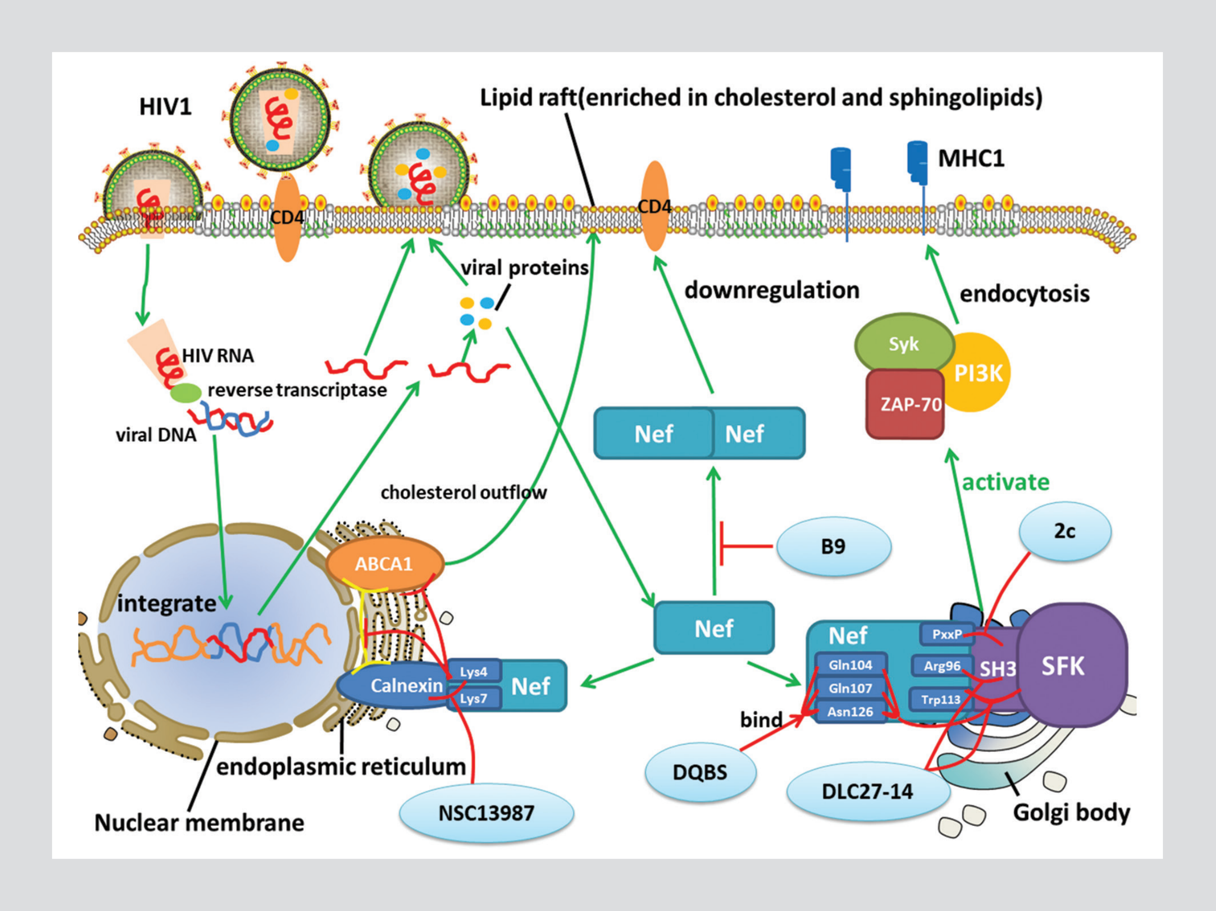

Figure 1. *Schematic demonstration of the working mechanisms of five Nef inhibitors.*

molecular docking technology was used to conduct structure-based virtual screening of the combined compound libraries and the binding affinities of the screened compounds toward Nef were ranked. The best hits from each library were selected for subsequent 30 ns molecular dynamics simulation and post-dynamic analysis. ZINC04177596 from the shape similarity-based ligand library and ZINC36617540 from the pharmacophore-based ligand library showed a better binding affinity than B9. Both hits were found capable of binding significantly to the dimeric core domain of Nef and were believed to inhibit the process of dimerization similarly to B9. In addition, above study also presented a set of structural criteria that may be critical for further optimization or design of novel Nef inhibitors.

Compound 2C: inhibitor of Nef-Src family tyrosine kinase (SFK) interaction

Another Nef-binding compound, the derivative of the natural product of Streptomyces known as 2c (2,4-dihydroxy-5-(1-methoxy-2-methylpropyl) benzene-1,3-dialdehyde) has also been reported significantly affecting Nef-dependent MHC-I downregulation[15] and viral infectivity.

It is suggested that 2c blocks Nef-dependent MHC-I downregulation in primary human cells by interfering with the assembly of SFK-ZAP-70/Syk-PI3K complex which starts the process of Nef-induced MHC-I downregulation. Nef-induced formation of SFK-ZAP-70/Syk-PI3K multi-kinase complex is initiated with the binding of PxxP SH3 domain of Nef to a trans-Golgi network-localized SFK, and followed by the activation of ZAP-70 and PI3K, which finally triggers the endocytosis of MHC-I molecules from the cell surface[16]. However, 2c had no effect on CD4 downregulation, which is instead mediated by a Class I PI3K-independent pathway[16].

In addition to above mechanism, studies showed that 2c interacted with Nef primarily through a cleft formed by the central β-sheet and the C-terminal α-helices of Nef. This cleft provided a good pocket for 2c. Besides, 2c was found capable of inducing a series of chemical shift changes in the polyproline helix of Nef that binds the RT loop of SH3 domain in SFKs, suggesting that 2c influences the conformation of the polyproline helix of Nef. Therefore, it is reasonable to speculate that 2c inhibits the interaction of Nef and SFK and then interferes with Nef-induced SFK activation either through allosteric blocking of SFK binding or by directly affecting the binding of the polyproline helix in Nef to SFKs or both. Although the binding site of compound 2c to Nef does not overlap with the structural features of Nef involved in SH3 binding and SFK recruitment, 2c was found reducing the infectivity of HIV-1 by its direct binding to Nef[17].

It is reported that overexpression of a mutant form of Hck whose SH3 domain lacks the linker region and kinase domain in viral producer cells results in the production of the viruses with reduced infectivity. 2c is confirmed to have an inhibitory effect on Nef-dependent Hck activation[18]. Moreover, 2c inhibits the binding of Hck to the PxxP domain of Nef, and the inhibitory effect is more significant when pre-incubation of the Nef-PxxP peptide with 2c occurs before its incubation with Hck. 2c was found disrupting Nef-Hck binding in a dose-dependent manner and also inhibiting the interaction of Nef with Lyn or Src[19]. At a low concentration, 2c impaired Nef-induced kinase activity of Hck while 2c at a high concentration directly inhibited Hck. Hence, the ability of 2c to differentially affect Nef-induced Hck activation suggests 2c bind Nef directly. This speculation is further supported by a computer-assisted simulation of the 2c-Nef docking model[20]. This model demonstrates that both the SH3 domain of Hck and 2c directly bind to Nef and that their binding sites overlap, supporting the idea that 2c reduces the infectivity of offspring viruses by perturbing Hck functioning. Compound 2c is, therefore, a valuable chemical probe to reveal the underlying molecular mechanisms by which Nef enhances the infectivity of HIV-1.

N-(3-aminoquinoxalin-2-yl)-4-chlorobenzenesulfonamide (DQBS): inhibiting Nef-mediated downregulation of MHC-I by preventing the assembly of SFK-ZAP-70/PI3K complex

Small molecules that interfere with Nef mediated downregulation of MHC-I may be powerful adjuvants to existing antiretroviral drugs to thwart viruses from evading immune strategies. Studies have revealed that the compound, a dihydrobenzo-1,4-dioxin-substituted analog, of DQBS potently blocked Nef-dependent HIV replication and MHC-I downregulation with an IC_{50} value of 130 nM. It significantly inhibited HIV replication at a concentration of 5 μM. HIV-1 caused Nef-dependent tyrosine phosphorylation of the activation loop of SFK, which was inhibited by approximately 50% in the presence of DQBS. The most favored binding site for DQBS is located at the dimer interface. The Nef residues which interact with DQBS have been identified and they are Gln104, Gln107, and Asn 126[21]. DQBS is predicted to make polar contacts with Asn126 plus an additional contact with Thr138. In addition, computational docking studies reveal that DQBS binding may affect the accessibility of Nef Trp113, which prevents the interaction of Nef with the SH3 domain of SFK and the PACS-2 trafficking protein that is critical to the assembly of SFK-Zap-70/ PI3K complex[15,16]. Zap-70 is implicated in HIV-1 replication and viral spread[22,23], suggesting that DQBS may interfere with HIV-1 replication by blocking Nef-dependent Zap-70 activation in T-cells. Inhibition of Zap-70 may also contribute to the antiretroviral efficacy of this compound. Further studies support a mechanism of action that is mediated through Nef and by directly affecting Zap-70 or Hck activity. By differential scanning fluorimetry assays, it was observed that DQBS could cause thermal destabilization of Nef protein in a concentration-dependent manner, thereby interfering with the recruitment and activation of SFKs and other effector proteins.

The anti-Nef activities of the compound DLC27-14, B9, and DQBS were compared at the concentration of 3 μM and the results showed that both DQBS and B9 reduced viral replication[21]. On the other hand, DLC27-14 was less potent with reducing viral replication by about 25% at this concentration. Compared to DQBS, 2c was less potent in both MHC-I downregulation and antiviral activity. This difference may be related to a weaker binding affinity of 2c for Nef and the possibility that DQBS may occupy a number of sites on the Nef structure that is important for MHC-I downregulation and viral growth[24].

1[(7-Oxo-7H-benz[de]anthracene-3-yl) amino]anthraquinone

As is proved in the experiment of Ruth Hunegnaw, 1[((7-Oxo-7H-benzo[anthracene]-3-yl]amino]anthraquinone] (NSC 13987) prevented the interaction of Nef and calnexin, reduced the abundance of and impaired the functional activity of ATP-binding cassette A1, so as to inhibit HIV-1 replication.

The previous studies have shown that HIV critically depends on host cholesterol metabolism to optimize viral replication[25-28]. The ATP-binding cassette A1 (ABCA1), a highly glycosylated transporter, has been proved to be able to inhibit HIV-1 replication by reducing the abundance of lipid rafts on the plasma membrane and thus affecting the production and infectivity of offspring viral particles[29]. It also means that HIV-1 can downregulate ABCA1 to facilitate its infection and replication. An important mechanism of downregulation of ABCA1 by HIV-1 is through Nef-Calnexin interaction. This interaction leads to ABCA1 retention in endoplasmic reticulum and disruption of calnexin binding to ABCA1. Nef-calnexin interaction that is mediated by the C-tail of calnexin and Lsy4 and Lsy7 of Nef disrupts calnexin-assisted maturation of ABCA1 and thus impairs cholesterol efflux. Based on above information, a screening was carried out for compounds that could destroy the interaction between Nef and calnexin, and 1[(7-OXO-7H-Benz[DE] Anthracene-3-YL] Amino] anthraquinone (NSC13987) was found capable of largely preventing calnexin and Nef interaction, reversing Nef-mediated downregulation of ABCA1 and restoring normal cholesterol outflow.

In addition, studies showed that compared with the control, the compound had a nearly 2-fold higher inhibitory effect on viral replication. On the one hand, this compound can prevent Nef from downregulating ABCA1. On the other hand, previous reports provided evidence that anthraquinone derivatives inhibited ribonuclease H function[16,30]. It can also prevent cholesterol metabolic disorders. If developed into drugs, this compound can simultaneously target both HIV and metabolic complications caused by the virus.

Perspective

Nef plays a critical role in HIV-1 life cycle, which makes it a rational target for antiretroviral drug development. Studies demonstrated that the structure of Nef inhibitors could be chemically modified without compromising their antagonistic effects[31]. This unique feature of Nef inhibitors together with their effects on inhibiting viral replication may contribute to the reduction of the size of HIV-1 viral reservoir. Therefore, HIV-1 Nef inhibitors provide a new type of armory for the treatment of HIV infection. Understanding the working mechanism of Nef is crucial for the design of potent Nef inhibitors, and a novel avenue toward the development of next generation of Nef inhibitors is coming to the horizon.

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