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Identification of an HIV-1 replication inhibitor which rescues host restriction factor APOBEC3G in Vif–APOBEC3G complex

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ABSTRACT

HIV-1 Vif protein is one of the most crucial accessory proteins for viral replication. It efficiently counteracts the important host restriction factor APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G, A3G) which is lethal to HIV-1 by causing G to A mutation of viral genome. Vif protein mediates degradation of APOBEC3G via the complicated protein–protein interactions of Vif, APOBEC3G, Elongin C/B and Cullin 5. The importance of Vif–APOBEC3G complex makes it a good potential target to develop new therapeutics of HIV-1. We identified a potent HIV-1 replication inhibitor (ZBMA-1, IC_{50} = 1.01 μM) that efficiently protected APOBEC3G protein by targeting Vif–APOBEC3G complex. The co-immunoprecipitation and docking studies indicated that compound ZBMA-1 affected the binding of Elongin C with Vif protein.

1. Introduction

Human immunodeficiency virus-1 (HIV-1), which still causes about 1.5 million deaths per year, has been a worldwide public health challenge for more than 30 years. The highly active antiretroviral therapy (HAART) that targets HIV-1 proteins including protease, integrase and transcriptase, has been successful in reducing viral counts. However, HAART has not been able to eradicate HIV-1 and has shortcomings including high cost, serious side effects and drug resistance (Mills et al., 2006; Richman et al., 2004; Ross et al., 2007). Discovering new target proteins or protein–protein complexes has been the focus towards developing new anti-HIV-1 therapeutics (Adamson and Freed, 2010). Various host-virus protein–protein interactions are crucial for HIV-1 virus in its life cycle. HIV-1 recruits host proteins to facilitate its entrance, transcription and assembly in the host cells and on the other hand host cells also develop defense mechanism to inhibit these key processes required for the viral replication in the host cells (Jager et al., 2012). APOBEC3G is one of the host restriction factors which can catalyze the hypermutation of viral genome from G to A, thus leading to highly efficient interruption of HIV-1 replication (Harris et al., 2003; Holmes et al., 2007; Lecossier et al., 2003; Mangeat et al., 2003; Suspene et al., 2004; Yu et al., 2004a; Zhang et al., 2003). To antagonize APOBEC3G's defense function, HIV-1 produces Vif protein to mediate proteasomal degradation of APOBEC3G via the ubiquitin pathway by exploiting Elongin C/B and Cullin 5 complex. (Gabuzda et al., 1992; Mariani et al., 2003; Marin et al., 2003; Mehle et al., 2004a,b; Sheehy et al., 2003; Stopak et al., 2003; Zhang et al., 2012). The key role of Vif against host defense protein APOBEC3G makes it a good target to develop new anti-HIV-1 therapeutics (Ali et al., 2012; Cen et al., 2010; Li et al., 2012; Nathans et al., 2008; Zuo et al., 2012).

Herein, we reported that a high-throughput screening (HTS) system was applied for a synthesized chemical library followed by the anti-HIV-replication assay. Compound ZBMA-1 with low cell toxicity was identified to protect APOBEC3G in the presence of Vif and thus possessing potent anti-HIV-1 replication activity (IC_{50} = 1.01 μM). Co-immunoprecipitation experiments, docking

Abbreviations: HIV-1, human immunodeficiency virus type-1; Vif, viral infectivity factor; A3G/APOBEC3G, (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G); ELISA, enzyme-linked immunosorbent assay; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

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studies and mutation experiments showed that compounds ZBMA-1 interrupted the interaction of Vif and Elongin C.

2. Materials and methods

2.1. General chemistry methods

All reagents and solvents were purchased from Sigma–Aldrich, J&K Chemical and Aladdin. Purity was analyzed by reverse-phase HPLC performed on the Agilent 6130 Quadrupole and Agilent 1200 equipment, or on the Agilent ProStar 218 system. NMR and HRMS were recorded at the Instrumental Analysis & Research Center in Sun Yat-sen University with a Thermo Scientific LTQ-Orbitrap Elite mass spectrometer.

2.2. Plasmids and cells

The sequence of full-length *vif* was amplified with PCR from an infectious HIV-1 clone pNL4-3 and inserted into pcDNA3.1-intron with a fused hemagglutinin (HA) tag at the N terminus. The cDNA fragment coding for hA3G was cloned into pEGFP-C1, as described previously (Liu et al., 2012). Human Elongin C, Cullin 5 and CBF-β with FLAG epitope tag sequences at their 5′termini were amplified through reverse transcription-polymerase chain reaction (RT-PCR) with the mRNA of 293T cells as the template. The accuracy was confirmed by DNA sequencing. The tagged Elongin C, Cullin 5 and CBF-β were then inserted into pcDNA3.1 vector. The Elongin C mutant sequences with FLAG epitope tag sequences at their 5′termini were amplified through PCR with the plasmid pcDNA3.1-Elongin C-Flag as the template. The primers′ sequences were as follows, the 5′primer: CGCGGATCCGCCACCATGGATTACA AGGATGACGATGACAAGGGCGGCGGCGATGGAGAGGAGAAAACCTA TG; the 3′primer for pcDNA3.1- Elongin C D111A-Flag: CCGCTCGAG TTAACAAGCTAAGAAGTTCGCAGCCATCA; the 3′primer for pcDNA3.1-Elongin C D111L-Flag: CCGCTCGAGTTAACAAAGTAA GAAGTTCGCAGCCATCA. The 293T cells were maintained in the conditioned Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), plus 100 μg/ml penicillin and streptomycin. The H9 and SupT1 cells were cultured in conditioned RPMI 1640 supplemented with 10% FBS and 100 μg/ml penicillin and streptomycin. (Liu et al., 2012) The SupT1-A3G cell line was generated by our lab as described previously (Pan et al., 2014).

2.3. High-throughput screening

Vif-HA and A3G-GFP plasmids were co-transfected into 293T cells in 96-well plate (Corning, Costar) and Lipofectamine 2000 (Invitrogen) by following the instructions of manufacturer. After transfection, a synthesized library was added with a Tecan Freedom EVO150 (Tecan, Männedorf, Schweiz) with a final concentration of 50 μM. Column 12 received only DMSO instead of any compound. In addition, column 1 was only transfected with the plasmid expressing A3G-GFP as a control. The GFP expression was detected with a PE Envision (Perkin-Elmer) at 48 h post-transfection.

2.4. HIV-1 production and infection

HIV-1 infectious clone, pNL4-3 (X4) was amplified with HB101 competent cells (Promega). To generate viruses, 293T cells were transfected with 10 μg of infectious clones and Lipofectamine 2000 (Invitrogen) by following the instructions of manufacturer. Culture supernatants were harvested at 48 h post-transfection and stored at −80 °C. To normalize viral inputs, the amount of p24 was measured by HIV-1 p24 enzyme-linked immunosorbent assay (ELISA). The target cells (1×10^6) were infected with the equivalent of 5 ng HIV-1 p24 in 1 ml for 3 h at 37 °C. The virus-containing supernatants were then removed by washing 3 times with PBS. The cells were maintained in conditioned RPMI 1640 medium and the HIV-1 replication was monitored by p24 detection (Zhang et al., 2003).

2.5. The preparation of PBMCs

The peripheral blood mononuclear cells (PBMCs) were isolated from healthy human donors through Ficoll gradient centrifugation and were cultured in the RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 100 units/ml of penicillin and 100 μg/ml of streptomycin (Invitrogen) and the PBMCs were activated by 1 μg/ml CD3 and 1 μg/ml CD28.

2.6. IC_{50} assay

H9 cells (1×10^6) were infected with the equivalent of 5 ng HIV-1 p24 antigen in 1 ml for 3 h at 37 °C. The virus-containing supernatants were then removed by washing 3 times with PBS. The cells were treated with compound ZBMA-1 in indicated concentrations and HIV-1 replication was monitored by p24 detection at day 4 post infection (Zhang et al., 2003).

2.7. Co-immunoprecipitation (Co-IP)

pcDNA3.1-Vif-HA was co-transfected with pcDNA3.1-A3G/EC-Flag and were lysed 48 h later with IP lysis buffer [150 mM NaCl, 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1% Triton X-100, and 0.5% NP-40]. Then the lysate was incubated with anti-HA beads (Sigma) overnight at 4 °C. Then IP products were centrifuged and washed 3 times with washing buffer. Western blot experiments were conducted to analyze the immunoprecipitated samples with the following primary antibodies: anti-FLAG (rabbit polyclonal, MBL) or anti-HA (mouse monoclonal, MBL) (Liu et al., 2012).

2.8. Virus infectivity

In the pseudotyped virus experiment, pNL4-3-Δenv-GFP or pNL4-3-Δvif&env-GFP was co-transfected with VSV-G, pcDNA-3.1-A3G-HA or pcDNA-3.1 into 293T cells and cultured with ZBMA-1 or DMSO. Culture supernatants were harvested at 48 h post-transfection. To normalize viral inputs, the amount of p24 was measured by HIV-1 p24 enzyme-linked immunosorbent assay (ELISA). Jurkat cells were infected with the equivalent of 75 ng HIV-1 p24. The virus infectivity was analyzed 24 h post-infection by flow cytometry. The 293T lysates were collected for Western blot analysis (Aires da Silva et al., 2004; Mehle et al., 2004b; Sheehy et al., 2002).

In the wild type virus experiments, the first infection was performed in H9 cells (1×10^6 for each well). The cells were infected with the equivalent of 10 ng HIV-1 p24 for 3 h and then cultured in fresh 1640 medium with compound ZBMA-1 or control DMSO for 4 days. The amount of p24 was measured by HIV-1 p24 ELISA. The equivalent of 5 ng HIV-1 p24 was taken out from the four experiment groups in the first infection respectively and infected 1×10^6 H9 cells for the second infection experiments. Then the H9 cells were cultured in fresh 1640 medium without ZBMA-1 for 4 days. The amount of p24 level was measured by HIV-1 p24 ELISA.