FEBS Letters 589 (2015) 2019–2025

FEBS Letters

journal homepage: www.FEBSLetters.org

ZNF10 inhibits HIV-1 LTR activity through interaction with NF-κB and Sp1 binding motifs

Hironori Nishitsuji [a,c,*], Leila Sawada [a], Ryuichi Sugiyama [a,d], Hiroshi Takaku [a,b,*]

[a] Department of Life and Environmental Sciences, Chiba Institute of Technology, 2-17-1 Tsudanuma, Narashino, Chiba 275-0016, Japan
[b] Research Institute, Chiba Institute of Technology, 2-17-1 Tsudanuma, Narashino, Chiba 275-0016, Japan
[c] Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Chiba 272-8516, Japan
[d] Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

ARTICLE INFO

Article history:
Received 2 March 2015
Revised 4 June 2015
Accepted 11 June 2015
Available online 19 June 2015

Edited by Ivan Sadowski

Keywords:
KRAB domain
Zinc finger protein
TRIM28
SETDB1
HP1-gamma
HIV-1 LTR

ABSTRACT

Kruppel-associated box-containing zinc finger (KRAB-ZNF) genes constitute the single largest gene family of transcriptional repressors in the genomes of higher organisms. In this study, we isolated 52 cDNA clones of KRAB-ZFPs from U1 cell lines and screened them to identify which were capable of regulating HIV-1 gene expression. We identified 5 KRAB-ZFPs that suppressed ⩾50% of HIV-1 LTR. Of the 5 identified KRAB-ZFPs, the expression of ZNF10 significantly enhanced the transcriptional repression activity of the LTR compared with other ZNFs. In addition, the depletion of endogenous ZNF10 led to the activation of HIV-1 LTR. The repressor activity of ZNF10 was required for TRIM28, SETDB1 and HP1-gamma binding. These results indicate that ZNF10 could be involved in a potent intrinsic antiretroviral defense.

1. Introduction

The cellular tropism of HIV-1 replication has recently been found to be controlled by the 5′ LTR of HIV-1 [1]. The HIV-1 LTR functions as a typical promoter, containing several different binding sites specific for cellular and viral regulatory proteins [2,3]. It is regulated not only by viral proteins (Tat, Nef, Vpu) but also by ubiquitously expressed host factors such as Sp1 and TFIID. Additionally, inducible transcription factors such as NF-κB, AP1, Sp1, NFAT, USF, and COUP play roles in LTR-driven gene expression. These inducible regulatory factors vary in response to stimuli such as hyperthermia, oxidative stress and infection [4–6]. Moreover, the Kruppel-associated box (KRAB) zinc finger proteins (ZNFs) have the potential to regulate HIV-1 gene expression. Several groups have reported that artificially engineered KRAB domain-containing zinc finger proteins that bind to HIV-1 sequences also induce proviral silencing [7–9]. Furthermore, host proteins such as OTK18 suppress HIV-1 Tat-induced LTR activation through the negative regulatory element (NRE) and ETS binding site (EBS). All of these LTR binding elements are important due to their therapeutic potential in the reactivation of HIV-1 in latently infected cells [10–12].

Reynolds et al. reported that a genetically engineered KRAB domain containing a C2H2-type zinc finger motif suppressed Tat-mediated HIV-1 LTR activity, thereby making it an attractive candidate for antiretroviral therapy [8]. In addition, endogenous OTK18, which contains 13 C_2H_2-type zinc finger motifs, inhibits HIV-1 replication. OTK18 was identified by differential display of mRNA from HIV-1-infected macrophages and was shown to interact with and suppress the NRE (−255/−238) and EBS (−150/−139) in the HIV-1 LTR [11,13]. More recently, we showed that ZNF350 (ZBRK1) is able to repress HIV-1 replication through binding the −145 to −126 region of the HIV-1 LTR and ZNF1 in conjunction with TRIM28 and HDAC2, thereby suppressing HIV-1 LTR-driven gene expression [14]. Interestingly, the methylation driven by SETDB1 did not influence this mechanism, which probably reflects the fact that each ZNF has a unique pattern of inhibition. TRIM28 (tripartite

Author contributions: H.N. and H.T.: conceived and supervised the study; H.N. and H.T.: designed experiments; H.N., L.S., and R.S.: performed experiments and analyzed data: H.T.: analyzed data. H.T. and H.N. wrote the manuscript.

* Corresponding authors at: Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Chiba 272-8516, Japan (H. Nishitsuji). Research Institute, Chiba Institute of Technology, 2-17-1 Tsudanuma, Narashino, Chiba 275-0016, Japan. Fax: +81 47 478 0407 (H. Takaku).

E-mail addresses: lbnishitsuji@hospk.ncgm.go.jp (H. Nishitsuji), hiroshi.takaku@it-chiba.ac.jp, hiroshi.takaku@p.chibakoudai.jp (H. Takaku).

http://dx.doi.org/10.1016/j.febslet.2015.06.013
0014-5793/

motif-containing protein 28, also known as KRAB-associated protein 1, KAP1) is a well-characterized transcriptional repressor. The inhibition mediated by TRIM28 was proven to suppress endogenous retroviruses by recruiting the H3K9 methyltransferase ESET (also called SETDB1 or KMT1E) and heterochromatin protein 1 (HP1) in mouse ES cells [15,16]. TRIM28-mediated gene-specific transcriptional repression requires a ZNF protein such as ZFP809 to directly recognize integrated viral DNA [17]. Furthermore, TRIM28 inhibits HIV-1 integration through a cellular pathway targeting acetylated IN [18].

In this study, we investigated the role of KRAB-zinc finger proteins in the transcriptional repression of HIV-1. We found that ZNF10 highly represses HIV LTR-mediated transcription compared with other ZNFs. We also show that ZNF10 in conjunction with TRIM28, SETDB1 and HP1-gamma suppresses HIV-1 LTR-mediated transcription.

2. Materials and methods

2.1. Plasmids

The details of the plasmid constructs used in this study are provided in the Supplementary Materials and Methods section.

2.2. Cells

293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. MT4 and U1 cells were grown in RPMI 1640 supplemented with L-glutamine (Sigma Aldrich, St. Louis, MO, USA), 10% fetal bovine serum (FBS) (Biosera, Nuaille, France) and 1% antibiotics (Sigma Aldrich). The cultures were maintained at 37 °C/5% CO_2. On day 4, the U1 cells were collected and centrifuged at 1000 rpm for 3 min. The supernatant was discarded, and RNA was extracted from the pellets using RNeasy Plus Mini kits (Qiagen, Venlo, Netherlans). The RNA concentrations were measured with a spectrophotometer (Thermo Scientific, Wilmington, MA, USA), and RNA quality (absence of RNA degradation) was assessed by gel electrophoresis. The levels of HIV-1 p24 antigen were determined using chemiluminescence enzyme immunoassays (CLEIAs) (Lumipulsef, FUJIREBIO, Tokyo, Japan).

2.3. Western blotting

The details of the western blotting analysis are provided in the Supplementary Materials and Methods section.

2.4. Small interfering RNA (siRNA)

KRAB-ZNF (ZNF10, ZNF566, ZNF333, ZNF561, and ZNF324) mRNA was analyzed, and specific Stealth RNAi™ predesigned siRNAs were ordered (Life Technologies, Gland Island, NY, USA). siTRIM28 and siSETDB1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and siHP1-gamma was purchased from Sigma–Aldrich.

2.5. Immunoprecipitation

293T cells were transfected with 0.5 μg of pHA-ZNF10 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). At 48 h post-transfection, the transfected cells were harvested and suspended in 0.5 ml lysis buffer (20 mM Tris–HCl pH 7.5, 250 mM NaCl, 1 mM EDTA, 5% glycerol, 1% TritonX-100). The cell lysates were centrifuged at 15000×*g* for 20 min at 4 °C, after which the supernatant was incubated with 1 μg of anti-TRIM28 antibody and 40 μl of protein G magnetic beads for 2 h at 4 °C. The beads were then washed with PBS containing 0.02% TritonX-100, and the immunocomplexes were eluted by boiling in 20 μl of 5× sample buffer and analyzed by SDS–PAGE and Western blotting.

2.6. Luciferase assay

The luciferase assay details are provided in the Supplementary Materials and Methods section.

2.7. Measurement of HIV-1 p24 antigen

The p24 antigen measurement details are provided in the Supplementary Materials and Methods section.

2.8. HIV-1 challenge and culture assay

The HIV-1 challenge and culture assay details are provided in the Supplementary Materials and Methods section.

3. Results and discussion

3.1. Screening results

We obtained the coding DNA sequences (CDSs) of 134 human ZNFs and queried the NCBI protein database. We then isolated 134 cDNAs encoding Kruppel-related zinc finger genes from a cell line latently infected with HIV-1 (U1). To evaluate the level of expression of the 134 ZNFs in U1 cells, we performed RT-PCR analysis. We observed no detectable mRNAs encoding 82 of the 134 ZNFs in U1 cells (data not shown). Therefore, we focused on the other 52 ZNFs as candidate transcriptional repressors of the HIV-1 LTR.

Next, we determined whether these 52 ZNFs could inhibit HIV-1 LTR promoter activity. 293T cells were co-transfected with the KRAB-ZNF expression vectors and with the HIV-1 LTR-driven luciferase reporter plasmid. As shown in Fig. 1, ZNF10, ZNF566, ZNF333, ZNF561, and ZNF324 significantly suppressed $\geqslant$50% HIV-1 LTR activity compared with the empty vector control. By contrast, ZNF416, ZNF115, and ZNF41 potentially acted as positive regulators of HIV-1, promoting LTR-driven transcription (Fig. 1). Mysliwiec et al. reported that ZFP496 functions as a transcriptional activator when it is tethered to DNA or when it is directly bound to the DNA-binding motif [19]. However, in this study we did not analyze the up-regulation of LTR-driven transcription. To further investigate the effect of KRAB-ZNF (ZNF10, ZNF566, ZNF333, ZNF561, and ZNF324)-mediated repression of the LTR, we used siRNAs specific for the five identified KRAB-ZNFs. The introduction of each specific siRNA induced efficient knockdown of expression of the corresponding protein (Fig. 2A). Knockdown of ZNF10 expression significantly enhanced the transcriptional activity of the LTR compared with the knockdown of other KRAB-ZNFs (Fig. 2B). The KRAB zinc finger protein 10 (ZNF10) contains two KRAB domains (A and B boxes) and nine zinc finger regions [20].

To evaluate the effect of ZNF10 in T cells, MT-4 cells were transduced with the control or ZNF10-specific short hairpin RNA (shRNA) vectors and then infected with HIV-1. Virus replication was then monitored by measuring the production of p24 in the supernatant every two days post-infection. The depletion of ZNF10 in MT-4 cells resulted in twofold more HIV-1 replication at four and six days post-infection (Fig. 2C). These results suggest that ZNF10 inhibits HIV-1 gene expression through transcriptional repression of the LTR.