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Original article

Tristetraprolin inhibits HIV-1 production by binding to genomic RNA

Masae Maeda [a,b,c], Hirofumi Sawa [c,d,e], Minoru Tobiume [a], Kenzo Tokunaga [a], Hideki Hasegawa [a], Takeshi Ichinohe [a], Tetsutaro Sata [a], Masami Moriyama [b], William W. Hall [f], Takeshi Kurata [a], Hidehiro Takahashi [a,*]

[a] *Department of Pathology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan*
[b] *Department of Microbiology and Immunology, School of Medicine, Keio University, Tokyo, Japan*
[c] *CREST, JST, Japan*
[d] *Department of Molecular Pathobiology, Hokkaido University Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan*
[e] *21st Century COE Program for Zoonosis, Hokkaido University, Sapporo, Japan*
[f] *Department of Medical Microbiology, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland*

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Abstract

HIV-1 genome has an AU-rich sequence and requires rapid nuclear export by Rev activity to prevent multiple splicing. HIV-1 infection occurs in activated $CD4^+$ T cells where the decay of mRNAs of cytokines and chemokines is regulated by the binding of AU-rich elements to the mRNA-destabilizing protein tristetraprolin. We here investigated the influence of tristetraprolin on the replication of HIV-1. Treatment of siRNA against tristetraprolin in a latently HIV-1 infected cell line increases HIV-1 production following stimulation. A chloramphenicol acetyltransferase and luciferase assay revealed that exogenous tristetraprolin reduced HIV-1 virion production and in contrast increased the multiply spliced products. Furthermore, quantitative RT–PCR analysis showed tristetraprolin increases the ratio of multiple-spliced RNAs to un-, single-spliced RNA. Moreover, an electrophoretic mobility shift assay showed that tristetraprolin binds to synthesized HIV-1 RNA with AU-rich sequence but not to RNA with less AU sequence. These results suggest that tristetraprolin is a regulator of HIV-1 replication and enhances splicing by direct binding to AU-rich sequence of HIV-1 RNAs.

Keywords: Human immunodeficiency virus type 1; Tristetraprolin; AU-rich element

1. Introduction

Expression of human immunodeficiency virus type 1 (HIV-1) genes is regulated by several posttranscriptional mechanisms, which include RNA splicing, stability, transport and translation. For example, HIV-1 RNA contains inhibitory sequences (INS) negatively regulating expression [1], which is also known to be AU-rich [2]. Replacement of AU residues of HIV-1 mRNA has been reported to result in a marked increase in expression of HIV-1 Gag, Pol, and Env proteins independent of the Crm1/Rev/Rev-responsive element (RRE) export pathway [3]. In addition, another nuclear export system, constitutive transport elements (CTE)/Tap, can substitute Rev/RRE [4], suggesting that effective nuclear export is required to complement the character of AU-rich RNA genome. As we have previously reported that HIV-1 RNA is fragmented

Abbreviations: HIV-1, human immunodeficiency virus type 1; TTP, tristetraprolin; ARE, AU-rich element; RRE, Rev-responsive element; nt, nucleotide; RT, reverse transcription; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; VSV-G, vesicular stomatitis virus envelope glycoprotein.

* Corresponding author. Tel.: +81 3 5285 1111; fax: +81 3 5285 1189.
E-mail address: htakahas@nih.go.jp (H. Takahashi).

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in viral particles and in vitro-synthesized HIV-1 RNA is cleaved between U and A or C and A [5], effective nuclear export of HIV-1 RNA by Crm1/Rev/RRE might be required to rescue a potentially unstable RNA genome, and the balance between RNA decay and nuclear export could be an important facet of HIV-1 replication and pathogenesis.

Whereas activated $CD4^+$ T cells are a primary target for productive HIV-1 infection [6], HIV-1 production does not occur in resting $CD4^+$ T cells because of a low level of reverse transcriptase activity [7]. In activated cells, including $CD4^+$ T cells that produce cytokines, chemokines and other proteins in response to inflammation and infection, mRNAs are degraded rapidly following transient activation [8]. These mRNAs contain the AU-rich element (ARE) in their 3′-untranslated region (3′UTR) that binds with tristetraprolin (TTP, also known as TIS11, Nup475, or G0S24) [9]. TTP is the prototype of a family of proteins that possess a pair of closely spaced zinc fingers of the CCCH class and are capable to binding AU-rich elements (ARE) in the 3′UTR and subsequent destruction of transcripts of pro-inflammatory mediators such as tumor necrosis factor α, granulocyte/macrophage colony stimulating factor, and cyclooxygenase 2 [10].

We propose the hypothesis that TTP might interact with the AU-rich regions of HIV-1 RNA and that the regulation of the HIV-1 RNA genome could play important roles in the post-transcriptional control of HIV-1 replication in activated or memory T cells. We show that TTP reduced HIV-1 virion production by enhancing multiple splicing through binding to HIV-1 AU-rich RNA.

2. Materials and methods

2.1. Cell culture

The human cell lines HEK293T and HeLa were maintained under an atmosphere of 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. U1 cells [11] were cultured in complete medium (RPMI 1640 medium supplemented with 10% fetal bovine serum).

2.2. RNAi inhibition

siRNA treatment was achieved using synthetic oligonucleotides which were purchased from Ambion. Sense sequence of TTP siRNA is CCC AUA AAU CAA UGG GCC Ctt (small capital displays deoxyribonucleic acid) and antisense is GGG CCC AUU GAU UUA UGG Gtg. Two rounds of siRNA treatments were performed. siRNA was transfected by using the electric transfection apparatus, amaxa in U1 cells.

2.3. Cloning, mutagenesis, and plasmid construction for TTP or rev expression vectors

Polyadenylated RNA was isolated from HeLa cells and subjected to reverse transcription (RT) with SuperScript $RNaseH^-$ reverse transcriptase (Invitrogen Life Technologies) and a 15-nucleotide poly(dT) primer. The coding region of human TTP cDNA was amplified by polymerase chain reaction (PCR) with the reverse-transcribed cDNA as the template and with the primers 5′-CGT GAATTC ATG GAT CTG ACT GCC ATC TAC GAG AGC CT-3′ (TTPF, *Eco*RI site underlined) and 5′-GAC CGG GCA G GCGGCCGC TCA CTC AGA AAC AGA GAT-3′ (TTPR, *Not*I site underlined). The PCR product was digested with *Eco*RI and *Not*I and then cloned into pcDNA4/HisMax-C (Invitrogen Life Technologies), a mammalian expression vector containing the Xpress epitope tag sequence, or into pCAGGS-IRES-EGFP [12], yielding pchTTPwt or pCAGGSTTP, respectively. The resulting plasmids were sequenced with a DNA sequencer (model 377A; Perkin Elmer, Norwalk, CT). Expression vectors for TTP deletion mutants, including 1–101, 76–189, and 176–320, were similarly constructed with pchTTPwt as the template and with the primers 5′-TCT CTG AG GCGGCCGC TTA TAG CTC AGT CTT GTA GCG CGA -3′ (R1–101), 5′-CTG GCT GAATTC CTG GGC CCT GAG CTG TCA CCCT-3′ (F76–189), 5′-AGG CCT GGT GCGGCCGC TTA GGT CCG GCG GCC AGA GGG CA-3′ (R76–189), and 5′-CCT GTG GAATTC CAG AGC ATC AGC TTC TCC GGC CT-3′ (F176–320). The resulting expression vectors were designated pcD1–101, pcD76–189, and pcD176–320, respectively.

For construction of vectors for Xpress-tagged Rev, the Rev cDNA was amplified by PCR with pSRαRev [13] as the template and with the primers 5′-AAA AAA AGATCT ATG GCA GGA AGA-3′ (Rev-*Bgl*II) and 5′-AAA AAAGTCGAC CTA TTC TTT AGTT-3′ (Rev-*Sal*I). The PCR product was digested with *Bgl*II and *Sal*I and then cloned into pEGFP-C1 (BD Biosciences Clontech, Palo Alto, CA) or pcDNA4/HisMax-C that had been digested with *Bam*HI and *Xho*I; the resulting vectors were designated pEGFP-Rev or pcMax-Rev.

2.4. Luciferase, chloramphenicol acetyltransferase (CAT), and p24 enzyme-linked immunosorbent (ELISA) assays

HEK293T cells were cotransfected with HIV-1 proviral DNA (pNL43 [14], pNL-Luc-E^-R^+ [15], or pNL-enCAT [16]) and TTP expression vectors (pchTTPwt, pcD1–101, pcD76–189, or pcD176–320) with the use of Fugene 6 (Roche, Mannheim, Germany) or by the calcium phosphate method. The amount of virus in culture supernatants was quantified by measurement of p24 antigen with a p24 Gag antigen capture ELISA assay (ZeptoMetrix, Buffalo, NY). For measurement of luciferase activity or CAT [17], cells were lysed and assayed with a Luciferase Assay System (Promega, Madison, WI) and a Lumat LB 96V luminometer (Perkin Elmer) or with a CAT ELISA (Roche).

Expression of wild-type and mutant TTP was examined by immunoprecipitation, SDS–polyacrylamide gel electrophoresis and immunoblot with mouse monoclonal antibodies to the Xpress epitope (Invitrogen) analysis or goat polyclonal antibodies to TTP (Santa Cruz Biotechnology, Santa Cruz, CA). The antibodies to Nef and to p24 also used for immunoblot analysis were kind gifts from Dr. Ikuta. Signals were obtained