Virology 522 (2018) 193–198

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

HIV-1 replication in CD4$^+$ T cells exploits the down-regulation of antiviral NEAT1 long non-coding RNAs following T cell activation

Hongbing Liu[a], Pei-Wen Hu[a], Jacob Couturier[b], Dorothy E. Lewis[b], Andrew P. Rice[a,*]

[a] Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA
[b] Department of Internal Medicine, University of Texas Health Science Center, Houston, TX, USA

ARTICLE INFO

Keywords:
HIV
NEAT1 lncRNA
Long non-coding RNA
Viral replication

ABSTRACT

The related NEAT1_1 and NEAT1_2 long noncoding RNAs (lnc RNAs) have been recently implicated in innate immunity against viral infection. We used CRISPR-Cas9 to generate Jurkat CD4$^+$ T cell lines with a knockout (KO) of the *NEAT1* gene. Viabilities of *NEAT1* KO Jurkat lines were indistinguishable from parental Jurkat cells, as was the induction of CD69 after T cell activation. The KO lines were however more sensitive to the induction of apoptosis than parental Jurkat cells. HIV-1 replication was higher in the KO lines than parental Jurkat cells, demonstrating an anti-HIV function of NEAT1 lncRNAs. We observed a strong down-regulation of NEAT1 lncRNAs following activation of resting peripheral blood mononuclear cells and purified CD4$^+$ T cells. These findings indicate that HIV-1 infection exploits the normal down-regulation of anti-viral NEAT1 lncRNAs in activated CD4$^+$ T cells to enhance viral replication.

1. Introduction

It has become appreciated in recent years that long noncoding RNAs (lncRNAs) have important functions in innate immunity (Zhang and Cao, 2016). Two such lncRNAs are the related NEAT1_1 and NEAT1_2 (Nuclear-Enriched Abundant Transcripts) lncRNAs. Both NEAT1 lncRNAs are transcribed from the same promoter but differ at their 3′ ends due to differential transcriptional termination (Wilusz, 2015). NEAT1_1 is 3.7 kb in length and is polyadenylated, while NEAT1_2 is 23 kb in length and is non-polyadenylated. NEAT1 lncRNAs are commonly up-regulated following viral infection, including infections of Japanese encephalitis virus, rabies virus (Saha et al., 2006), HSV, influenza virus (Imamura et al., 2014), hantavirus (Ma et al., 2017), and HIV-1 (Zhang et al., 2013). SiRNA depletions of NEAT1 lncRNAs enhance hantavirus replication (Ma et al., 2017), KSHV replication (Morchikh et al., 2017) and HIV-1 gene expression and replication (Budhiraja et al., 2015; Zhang et al., 2013), suggesting broad antiviral activity of these lncRNAs.

NEAT1 lncRNAs are found in two distinct nuclear structures – paraspeckles and a recently described ribonucleoprotein (RNP) complex termed the HDP-RNP. Paraspeckles are formed at the sites of transcription of the *NEAT1* gene where ~ 40 proteins use NEAT1 lncRNAs as scaffolds to assemble dynamic RNP structures with diameters of 0.5–1.0 μM (Fox et al., 2017; Mao et al., 2011). The HDP-RNP contains HEXIMI1, DNA-PK subunits (DNAPKc, Ku70, and Ku80) and additional proteins also found in paraspeckles. The assembly of NEAT1 lncRNAs in these RNPs is likely to underlie the antiviral activities of the lncRNAs.

A mechanism whereby paraspeckle-associated NEAT1 lncRNAs exert antiviral activity is through sequestration of a paraspeckle protein termed SFPQ. SFPQ is a transcriptional repressor of the *IL8* gene and induction of NEAT1 lncRNAs following influenza virus infection increases the number and size of paraspeckles, leading to sequestration of SFPQ in these nuclear structures, away from the *IL8* promoter and the resultant induction of *IL8* transcription (Imamura et al., 2014). SFPQ has been proposed to be a repressor of two additional antiviral genes, *RIG-I* and *DDX60*. Hantavirus infection induces NEAT1 lncRNAs and therefore paraspeckles, and this is associated with induction of RIG-1 and DDX60 mRNAs and proteins, presumably through sequestration of SFPQ from the *RIG-I* and *DDX60* promoters (Ma et al., 2017). The NEAT1 lncRNA HDP-RNP complex is involved in the recognition of infections by DNA viruses through the cGAS-STING-IRF3 pathway (Morchikh et al., 2017). The HDP-RNP associates with cGAS and its partner PQBP1. Upon recognition of viral DNA following KSHV infection, the HDP-RNP-cGAS-PQBP1 complex is remodeled, leading to activation of the cGAS-STING antiviral pathway.

The generation of a *NEAT1* knockout (KO) mouse line indicates that NEAT1 lncRNAs are not essential for cell viability (Nakagawa et al.,

* Correspondence to: Department of Molecular Virology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.
E-mail address: arice@bcm.edu (A.P. Rice).

https://doi.org/10.1016/j.virol.2018.07.020
Received 31 May 2018; Received in revised form 14 July 2018; Accepted 14 July 2018

2011). However, fertility of the KO mice is impaired due to a defect in formation of the corpus luteum (Nakagawa et al., 2014) and mouse embryo fibroblasts (MEFs) from *NEAT1* KO mice are more sensitive to induction of apoptosis than MEFs from wild type mice (Hirose et al., 2014). The role of NEAT1 lncRNAs in immune system function, either adaptive or innate, has not been examined in the *NEAT1* KO mice.

To investigate NEAT1 lncRNAs and HIV-1 infection, we generated *NEAT1* KO Jurkat CD4$^+$ T cell lines. Although the KO Jurkat cell lines were more sensitive than parental Jurkat cells to the induction of apoptosis by cycloheximide treatment, the KO lines possess growth rates and respond to T cell activation similar to parental cells. We found that HIV-1 replicates to higher levels in the KO lines than parental Jurkat cells, demonstrating an anti-HIV function in Jurkat CD4$^+$ T cells. We also observed that NEAT1 lncRNA levels are strongly down-regulated following activation of peripheral blood lymphocytes (PBMCs) and resting CD4$^+$ T cells isolated from blood donors. Productive HIV-1 infection in CD4$^+$ T cells requires cellular activation (Zack et al., 1990). Our findings indicate that the down-regulation of the antiviral NEAT1 lncRNAs in activated CD4$^+$ T cells contributes to high levels of HIV-1 replication in these cells.

2. Materials and methods

2.1. Cell lines

Jurkat and its derivatives (J3C9, J3E5) were cultured in RPMI supplemented with 10% fetal bovine serum (FBS); 293 T cells were cultured in DMEM supplemented with 10% FBS.

2.2. Primary cells

Resting CD4 T cells were isolated from healthy donors (Gulf Coast Regional Blood Center, Houston, TX) using the RosetteSep human CD4$^+$T cell enrichment cocktail (STEMCELL technologies, catalogue number (cat. no. 15062); activated cells were removed using CD30 Microbeads (Miltenyi Biotec, cat. no. 130-051-401). Cells were activated with phytohemagglutinin (PHA) (1 μg/ml) and cultured in RPMI supplemented with 10% fetal bovine serum. PBMCs from HIV-positive donors were obtained from a specimen bank established by Dr. Roberto Arduino (UT Houston Health Science Center). The cryopreserved PBMCs were thawed and half were left untreated, while the other half was treated with 1 μg/ml PHA for 2 days. Purified CD4$^+$ T cells and PBMCs were cultures in RPMI supplemented with 10% FBS and IL-2 (30 U/ml).

2.3. RNA isolation

Cytoplasmic RNA was isolated from cultured cells by the PARIS™ Kit Protein and RNA isolation system (Thermo Fisher Scientific, cat. no. AM1921) Total RNA was isolated from cultured cells using miRNeasy Mini Kit (Qiagen, cat. no. 217004), and Real-time RT-qPCR was performed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat. no. 4368814) and Powerup SYBR Green Master Mix (Applied Biosystem, cat. no. A25743) using primers for unspliced Gag and spliced viral RNAs, and pre-GAPDH and processed GAPDH RNAs as controls. Primers for unspliced viral RNA: Gag1-qPCR 5′ GGGACCCA GCCATAAAGC3′; Gag2-qPCR 5′ GCTGAATTTGTTACTTGGCTCA 3′; Primers for spliced viral RNA: Spliced-F 5′ GTCTCTCTGGTTAGACCAG 3′; Spliced-R 5′ TTGGGAGGTGGGTTGCTTTGATAGAG 3′; Primers for unspliced GAPDH: Pre-GAPDH-F 5′ CCACCAACTGCTTAGCACC 3′ Pre-GAPDH-R 5′CTCCCCACCTTGAAAGGAAAT 3′

2.4. HIV-1 NL4-3 virus production and infection

pNL4-3 plasmid (AIDS Reagent Program, cat. no. 114) was transfected into 293 T cell using lipofectamine 2000 (Thermo Fisher Scientific, cat. no.52758). Viruses were collected 48 h post-transfection and 0.45 μm filter filtered viruses were transduced into Jurkat cells in the presence of polybrene.

2.5. Antibodies

Goat-anti-HIV gp160B (cat. no.188), mouse-anti-HIV-1 gag (cat. no. 3539), and rabbit-anti-Rev (cat. no. 2949) were obtained from the NIH AIDS Reagent Program. Rabbit anti RBM14 (cat. no. ab70636) was from Abcam, rabbit-anti-PARP-1 (cat. no. 9542p) was from Cell Signaling, p54nrb (cat. no. 611278) was from BD Transduction Laboratories and β-actin (cat. no. 611278 A2103) from Sigma-Aldrich.

2.6. Immunoblots

Cells were lysed in RIPA buffer (50 mM Tris. Cl pH8, 150 mM NaCl, 1%NP-40, 0.5%DOC, 0.1%SDS) plus 5 mM DTT and protease inhibitor cocktail (Sigma-Aldrich cat. no. P8340) on ice for 30 min. The soluble portion of extracts was loaded on an SDS-PAGE followed by transfer to nitrocellulose membranes. After blocking with 5% BSA, membranes were probed with antibodies using standard protocols.

2.7. CRISPR-CAS9 knockouts

A Multiplex CRISPR/Cas9 Assembly System kit (Addgene kit 10000000055) was used to make *NEAT1* KO cell lines. Six guide RNAs (gRNAs) were designed with web-based software (two guide RNAs at the promoter region, two at the 5′ end and two at the 3′ end of NEAT1_2 sequences). Location of the gRNAs is shown in Fig. 1. Six gRNA expression cassettes were inserted into pX330A-1x6. The resulting plasmid together with a GFP expression plasmid was transfected into Jurkat cells by electroporation at 1600V for 10 ms for three times. After

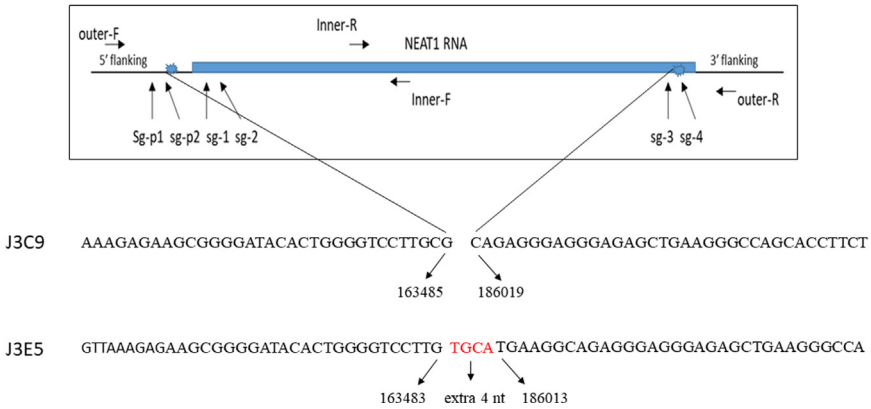

Fig. 1. Strategy to delete *NEAT1* gene. Positions of guide RNAs used to delete the *NEAT1* gene are indicated. Positions of PCR primers to verify deletion of *NEAT1* gene in genomic DNA are indicated (primer sequences are indicated in Materials and Methods). The genomic sequence of deletions for the J3C9 and J3E5 lines are shown at the bottom of the figure; deletions in both *NEAT1* alleles in both cell lines were identical. The sequence reference numbers are from Homo sapiens genomic DNA, chromosome 11: cloneRP11-86708.