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HTLV-1 Tax impairs K63-linked ubiquitination of STING to evade host innate immunity

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ABSTRACT

The cellular antiviral innate immune system is essential for host defense and viruses have evolved a variety of strategies to evade the innate immunity. Human T lymphotropic virus type 1 (HTLV-1) belongs to the deltaretrovirus family and it can establish persistent infection in human beings for many years. However, how this virus evades the host innate immune responses remains unclear. Here we report a new strategy used by HTLV-1 to block innate immune responses. We observed that stimulator of interferon genes (STING) limited HTLV-1 protein expression and was critical to HTLV-1 reverse transcription intermediate (RTI) ssDNA90 triggered interferon (IFN)-β production in phorbol12-myristate13-acetate (PMA)-differentiated THP1 (PMA-THP1) cells. The HTLV-1 protein Tax inhibited STING overexpression induced transcriptional activation of IFN-β. Tax also impaired poly(dA:dT), interferon stimulatory DNA (ISD) or cyclic GMP-AMP (cGAMP) –stimulated IFN-β production, which was dependent on STING activation. Coimmunoprecipitation assays and confocal microscopy indicated that Tax was associated with STING in the same complex. Mechanistic studies suggested that Tax decreased the K63-linked ubiquitination of STING and disrupted the interactions between STING and TANK-binding kinase 1 (TBK1). These findings may shed more light on the molecular mechanisms underlying HTLV-1 infection.

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1. Introduction

The innate immune system functions as the first line of host defense against viral invasion. The initiation of innate immune responses relies on the recognition of pathogen-associated molecular patterns (PAMPs) by an array of pattern-recognition receptors (PRRs)(Broz and Monack, 2013). Different forms of DNA derived from viruses can act as PAMPs to induce activation of signaling pathways, leading to the production of type I interferon (IFN-I) and other antiviral innate immune responses(Keating et al., 2011). So far, several molecules have been identified as sensors for viral DNA, including DNA-dependent activator of IFN regulatory factors (DAI), RNA polymerase III (RNA-Pol III), IFN-gamma inducible factor 16 (IFI16), DExD/H-box helicase 41 (DDX41), Ku70, stimulator of IFN genes (STING) and cyclic GMP-AMP synthase (cGAS)(Holm et al., 2013; Orzalli and Knipe, 2014; Paludan and Bowie, 2013).

Among these DNA sensors, STING, also known as MITA/MPYS/ERIS, has emerged as central mediator in the cytosolic DNA-induced signaling pathways, either being an adaptor or directly sensing cytosolic dinucleotides(Abe et al., 2013; Ouyang et al., 2012; Zhang et al., 2013). Other DNA sensors, such as IFI16 and DDX41, interact with STING and trigger the transcription of IFN-I(Burdette and Vance, 2013). Another DNA sensor, cGAS, produces the second messenger cyclic GMP-AMP (cGAMP) after binding to cytosolic dsDNA, which is subsequently recognized by STING via direct binding, leading to IFN-I induction(Cai et al., 2014). Following activation, STING dimerizes and translocates from endoplasmic reticulum (ER), through the Golgi apparatus, and to the perinuclear microsome compartment where it engages

Abbreviations: PAMPs, pathogen associated molecular patterns; PRRs, pattern-recognition receptors; IFN-I, type I interferon; STING, stimulator of IFN gene; DAI, DNA-dependent activator of IFN regulatory factors; RNA-Pol III, RNA polymerase III; IFI16, IFN-gamma inducible factor 16; DDX41, DExD/H-box helicase 41; cGAS, cyclic GMP-AMP synthase; cGAMP, cyclic GMP-AMP; ER, endoplasmic reticulum; TBK1, TANK-binding kinase 1; IKKi, inducible IkB kinase; IRF3/7, interferon regulatory factor 3/7; TRIM32/56, tripartite motif protein32/56; CREB, cyclic AMP responsive binding protein; NF-κB, nuclear factor kappa-B; TRIF, TIR domain-containing adaptor-inducing IFN-β; RIP1, receptor-interacting protein kinase 1; RTI, reverse transcription intermediate; HTLV-1, human T lymphotropic virus type 1; ATL, adult T-cell leukemia/lymphoma; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; PMA-THP1, phorbol12-myristate13-acetate (PMA)-differentiated THP1 cells; AZT, azidothymidine; CHX, cycloheximide; ISD, interferon stimulatory DNA.

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in TANK-binding kinase 1 (TBK1) binding. The STING-TBK1 complex is required for TBK1 activation, which subsequently recruits and activates the transcriptional factor interferon regulatory factor (IRF) 3 (Barber, 2011; Tanaka and Chen, 2012). During the process, tripartite motif protein (TRIM) 32 and TRIM56-mediated K63-linked polyubiquitination of STING plays a critical role, which is a prerequisite for TBK1 recruitment and STING-triggered IFN-I induction (Tsuchida et al., 2010; Zhang et al., 2012).

Retroviruses can trigger innate immune responses through DNA sensors (van Montfoort et al., 2014). There is an essential reverse transcription (RT) step in the life cycle of HIV-1 and other lentiviruses, leading to the production of a cDNA strand. The cDNA strand is used as a template to generate a proviral genome, which is then integrated into the genome of host cells. During this process, viral-derived DNA fragments accumulate in the cytosol, which can be recognized by the DNA sensors IFI16 and cGAS. IFI16 and cGAS interact with reverse transcription intermediates (RTIs) and recruit STING for downstream signal transduction (Gao et al., 2013; Jakobsen et al., 2013).

Human T lymphotropic virus type 1 (HTLV-1) belongs to the deltaretrovirus family, which has been linked to multiple diseases, including the aggressive blood cancer, adult T-cell leukemia/lymphoma (ATL)(Ishitsuka and Tamura, 2014), and the chronic, progressive neurological and inflammatory disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)(Fuzii et al., 2014; Ishitsuka and Tamura, 2014; Yamano and Sato, 2012). The total number of global HTLV-1 infected people is very uncertain, because the widely used number of 10–20 million infected people is estimated from studies about 20 years before, with incomplete epidemiological studies in many endemic areas(Gessain and Cassar, 2012). It is not well understood how HTLV-1 causes diverse clinical diseases and why these diseases are caused many years after initial infection. One explanation is that the virus has evolved effective mechanisms to evade host antiviral immune responses. So, it is quite meaningful to explore the exact mechanisms and this may provide clues for treatment of HTLV-1 associated diseases.

Although the exact cytosolic sensors for HTLV-1 remain unknown, it has been reported that RTI plays a role in triggering antiviral responses in HTLV-1 infected monocytes. A 90-nucleotide RTI from the U5 region of HTLV-1(ssDNA90), which was transfected into monocytes, stimulated antiviral responses in a STING-IRF3-dependent manner, suggesting the important role of STING in HTLV-1 induced antiviral innate immunity(Sze et al., 2013).

HTLV-1 encodes a critical transactivator, Tax, which plays a key role in promoting viral spread and inducing T cell transformation through multiple mechanisms, including activating some effectors, such as the cyclic AMP responsive binding protein (CREB) and CBP/p300, nuclear factor kappa-B (NF-κB) and so on(Currer et al., 2012; Matsuoka and Jeang, 2007). Given the fact that IFN-I restricts HTLV-1 replication(Cachat et al., 2013; Kinpara et al., 2009) and HTLV-1 has evolved some methods to evade this restriction(Colisson et al., 2010; Feng and Ratner, 2008; Journo and Mahieux, 2011), it is reasonable to explore the role of Tax in IFN-I production and innate immune responses. However, the role of Tax on IFN-I production is controversial now. One group reported that Tax suppressed viral RNA triggered innate immune signaling pathways by interacting with TIR domain-containing adaptor-inducing IFN-β (TRIF) and receptor-interacting protein kinase (RIP) 1 to disrupt IRF7 activation(Hyun et al., 2015), whereas another article suggested that Tax could be recruited into the TBK1/IKKi complex as a scaffolding-adaptor protein to enhance IFN-I gene expression(Diani et al., 2015).

In this study, we demonstrated that STING inhibited HTLV-1 protein expression in HTLV-1 infected PMA-THP1 cells and HTLV-1 protein Tax inhibited viral DNA triggered IFN-I production targeting STING. Tax interacted with STING and decreased its K63-linked ubiquitination, leading to reduced STING-TBK1 association and decreased IFN-I production. This work may uncover one of the mechanisms utilized by HTLV-1 to escape from innate immunity.

2. Materials and methods

2.1. cDNA constructs and reagents

Human cGAS and TBK1 were amplified by PCR using cDNA from HSV-1 infected HEK293 cells and were subsequently cloned into a pcDNA3.1-Flag/HA vector (Invitrogen). HTLV-1 protein Tax was amplified by PCR using cDNA from MT2 cells (HTLV-1 transformed T cell line). HA-Ubi, pNF-κB-Luc, pISRE-Luc, pIFN-β-Luc, Flag-STING and its deletion mutants were obtained as described previously (Wang et al., 2013; Wang et al., 2015). The anti-HA antibody was obtained from Covance (HA.11; 16B2; CO-MMS-101R), and the anti-Flag (M2; F3165) antibody was purchased from Sigma-Aldrich. The anti-STING antibody (19851-1-AP) and β-actin (60008-1-Ig) antibody were purchased from Proteintech. The anti-IRF3 antibody (sc-9082), anti-Tax antibody (sc-57872) and anti-Ubi (sc-8017) were obtained from Santa Cruz Biotechnology. The antibody specific for IRF3 phosphorylation at residue Ser396 (4947), the anti-p65 antibody (4764), and the phospho-p65 (Ser536) antibody (3033) were purchased from Cell Signaling Technology. The anti-HTLV-1-p19 (ab9080) antibody was obtained from Abcam. Poly(dA:dT) (tlrl-patn) was obtained from InvivoGen. PMA (S1819) was obtained from Beyotime. The 90-base-long HTLV-1 ssDNA90 is the reverse complement of the 5′UTR region (315–404) of complete HTLV-1 genome (NCBI) and was synthesized from the Sangon Biotech. The sequence was as follows: 5′-CTGTGTACTAAATTTCTCTCCTGGAGAGTGCTATAGA-ATGGGCTGTCGCTGGCTCCGAGCCAGCAGAGTTGCCGGTACTTGG-CCGTGGGC-3′. The scrambled ssDNA90 as a control was also synthesized from the Sangon Biotech. The sequence was as follows: 5′-ATTCAGCTCACGGCGTCGAGTGCTGCTCGATGGCTCCTTAG-TCCTGCTAAGTCGAGGTGGCTAATCCGGTAGTCGGTCGGATGGAA-TTCG-3′.

2.2. Cell culture, transfection and stimulation

HEK293T and HEK293 cells were cultured in DMEM. MT2, MT4 and THP1 cells were grown in RPMI 1640. All cells were supplemented with 10% FBS (Invitrogen), 4 mM/L-glutamine, 100U/ml penicillin, and 100U/ml streptomycin under humidified conditions with 5% CO_2 at 37 °C. Transfection of HEK293T cells, HEK293 cells and THP1 cells was performed with Lipofectamine 2000 (Invitrogen). For stimulation, poly(dA:dT) (1 μg/ml) and ssDNA90 (0.5 μg/ml) were delivered into cells using Lipofectamine 2000.

2.3. Immunoprecipitation and immunoblot analysis

Immunoprecipitation and immunoblot analysis were performed as described previously (Wang et al., 2013). In short, either HEK293T or HEK293 cells were transfected with various combinations of plasmids. At 24 h after the transfection, the cell lysates were prepared in lysis buffer containing 1.0% (vol/vol) Nonidet P40, 20 mM Tris-HCl, pH 8.0, 10%(vol/vol) glycerol, 150 mM NaCl, 0.2 mM Na_3VO_4, 1 mM NaF, 0.1 mM sodium pyrophosphate and a protease inhibitor 'cocktail' (Roche). After centrifugation for 20 min at 14,000g, supernatants were collected and incubated with the indicated antibody together with protein A/G Plus-agarose immunoprecipitation reagent (Santa Cruz Biotechnology) at 4 °C for 3 h or overnight. After three washes, the immunoprecipitates were boiled in SDS sample buffer for 10 min and analyzed by immunoblot. For endogenous coimmunoprecipitation experiments, MT2 cells were

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