Molecular Immunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Molecular Immunology

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

Activation of NF-κB induced by TRIMCyp showing a discrepancy between owl monkey and northern pig-tailed macaque

Jia-Wu Zhu[a], Dan Mu[a], Feng-Liang Liu[a], Meng-Ting Luo[a,b], Rong-Hua Luo[a], Yong-Tang Zheng[a,b,c,*]

[a] Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences/Key Laboratory of Bioactive Peptides of Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
[b] Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China
[c] The National Kunming High Level Biosafety Research Center for Nonhuman Primate, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China

ARTICLE INFO

Keywords:
TRIMCyp
HIV-1
NF-κB
AP-1
IFN-β

ABSTRACT

TRIMCyp generated by retrotransposition of a *cyclophilin A* inserting into *TRIM5* locus, has been identified in owl monkey and most of Old World monkeys (OWM). Owl monkey TRIMCyp (omTRIMCyp) inhibits HIV-1 infection by direct interaction with viral capsid and indirect innate immune induction, whereas most of TRIMCyps from OWM cannot inhibit HIV-1, and the impact of which on immunoregulation is largely unknown. Here we reported that omTRIMCyp induces NF-κB, AP-1 and IFN-β activation in a dose-dependent manner, while TRIMCyp from northern pig-tailed macaque (npmTRIMCyp) does not activate NF-κB and moderately enhances AP-1 and IFN-β activities. The Cyclophilin A (CypA) domain plays an important role in omTRIMCyp-mediated NF-κB activation, and RBCC domains have a synergetic effect. We further indicated the mechanism by which npmTRIMCyp unable to activate NF-κB is that npmTRIMCyp hardly phosphorylates IκBα, different from omTRIMCyp which dramatically induces IκBα phosphorylation. Ubiquitination activity of omTRIMCyp was greater than npmTRIMCyp, although both could be ubiquitylated. Given that npmTRIMCyp neither interacts with viral capsid resulting in susceptibility to HIV-1 infection, nor activates NF-κB that is indispensable to HIV-1 provirus transcription, we proposed a model that npmTRIMCyp may play an important role in HIV-1 infected northern pig-tailed macaque with latency.

1. Introduction

Intrinsic immunity refers to a set of recently identified cellular-based antiretroviral defense mechanisms, which combines aspects of innate and adaptive immunity. Intrinsic immunity comprises cellular proteins that constitutively express and can bind to viral components directly, allowing viral infection to be halted before the onset of interferon (IFN) response, although the expression of these proteins also can be further induced by IFN to enhance the antiviral activity (Bieniasz, 2004; Yan and Chen, 2012). Host restriction factors form part of intrinsic immune system and play an important role in limiting cross-species transmission of HIV-1 and other retroviruses (Yan and Chen, 2012).

TRIM5α, an extensively studied cytoplasmic protein, is one of large tripartite motif (TRIM) family of proteins whose N-terminal contain a RING finger domain, one or two B-box domains and a coiled-coil domain (collectively known as RBCC motif), linked to a variable C-terminal domain, which in the case of TRIM5α is a B30.2/SPRY domain (Stremlau et al., 2004). More than 70 TRIM proteins have been identified in humans, most of which are involved in a variety of cellular processes, including transcriptional and post-translational regulation, cell growth and differentiation, and immune regulation (Rajsbaum et al., 2014; Turrini et al., 2014). The eminent role of TRIM5α was unveiled as the discovery of its antiretroviral activity, which potently inhibits HIV-1 and other retroviruses through direct recognition of the retroviral capsid (CA) via the B30.2/SPRY domain in the cytoplasm of target cells at an early post-entry step, resulting in a premature uncoating and abortive infection in a species-specific manner (Perron

Abbreviations: TRIM, tripartite motif; OWM, Old World monkeys; omTRIMCyp, owl monkey TRIMCyp; npmTRIMCyp, northern pig-tailed macaques; CypA, cyclophilin A; HA, hemagglutinin; CA, capsid; Ub, ubiquitin; TAK1, transforming growth factor-β-activated kinase 1

* Corresponding author at: Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China.
E-mail address: zhengyt@mail.kiz.ac.cn (Y.-T. Zheng).

https://doi.org/10.1016/j.molimm.2018.08.001
Received 26 March 2018; Received in revised form 30 July 2018; Accepted 1 August 2018

et al., 2004; Stremlau et al., 2004, 2006; Yap et al., 2004). The block to HIV-1 infection conferred by TRIM5α in Old World monkeys (OWM) occurs before reverse transcription and is dependent on the sensitivity to HIV-1 CA (Stremlau et al., 2004).

Soon after discovery of TRIM5α, a very interesting fusion protein TRIMCyp, was also found to be sufficient to confer potent resistance to HIV-1 infection in owl monkey species (*Aotustrivirgatus* and *Aotusnancymaae*). Owl monkey TRIMCyp (omTRIMCyp) was generated by exon shuffling via a long interspersed nuclear element (LINE)-1-mediated retrotransposition of a *cyclophilin A (CypA)* complementary DNA inserting into intron 7 of *TRIM5* locus (Nisole et al., 2004; Sayah et al., 2004). Although the process of retrotransposition mediated by LINE-1 presumably results in generation of pseudogenes, omTRIMCyp containing the intact CypA domain possesses potent anti-retroviral activities, allowing a good example of gain-of-function by retrotransposition. Besides the conserved N-terminal RBCC domains, omTRIMCyp presents a CypA domain at C-terminus that almost precisely replaces the B30.2/SPRY domain of TRIM5α (Nisole et al., 2004). As restriction specificity of TRIM5α maps to the B30.2/SPRY domain, the CypA domain of omTRIMCyp acts on recognizing the incoming HIV-1 CA protein and promoting its premature disassembly. It therefore seems likely that the B30.2/SPRY domain of TRIM5α or the CypA domain of omTRIMCyp is responsible for restriction specificity, though RBCC domains are also required for inhibition of infection.

After that, we and other groups discovered another novel pattern of CypA insertion in OWM orthologs including pig-tailed macaque (*Macaca leonine* and *Macaca nemestrina*), rhesus macaque (*Macaca mulatta*), cynomolgus macaque (*Macaca fascicularis*) and assam macaque (*Macaca assamenis*) (Brennan et al., 2008; Cao et al., 2011; Kuang et al., 2009; Liao et al., 2007; Liu et al., 2015; Mu et al., 2018; Newman et al., 2008; Virgen et al., 2008; Wilson et al., 2008). Of these OWMs, the *CypA* gene is inserted at the 3'-untranslated region (3'-UTR) of the *TRIM5* locus, which differs from that of the owl monkey species. Due to the different insertion, the *TRIM5* intron 6 generates a G-to-T mutation at the 3' splice site, thereby leading to loss of *TRIM5* exon 7 to encode the central domain of the Linker2 region (Kuang et al., 2015; Liu et al., 2015). TRIMCyp fusion protein from pig-tailed macaque, like omTRIMCp, is totally homozygous, but shows little restriction of HIV-1 replication, as a result of a point mutation in the CypA domain that leads to the failure of CypA-CA interaction (Liu et al., 2015; Virgen et al., 2008). In addition to the well-characterized function in direct restricting infections by the retroviruses, recent work has demonstrated that TRIM5 proteins including human TRIM5α and omTRIMCyp are supposed to be a pattern recognition receptor (PRR) upon binding to susceptible retroviral CA, in that it generates unanchored K63-linked polyubiquitin (polyUb) chains which functionally activate transforming growth factor-β-activated kinase 1 (TAK1) complex, and in turn stimulate NF-κB and AP-1 signaling, contributing to promoting the secretion of type I interferon (Pertel et al., 2011). However, the impact of TRIMCyp from OWM orthologs on the regulation of innate immune signaling pathways has remained completely uncharacterized.

Toward this end, we set out to investigate the effect of TRIMCyp from northern pig-tailed macaques (npmTRIMCyp) on the activation of NF-κB, AP-1 and IFN-β signaling. In agreement with findings from the previous study by Pertel and colleagues (Pertel et al., 2011), we found that the ectopic expression of omTRIMCyp significantly stimulated activation of NF-κB- and AP-1-responsive promoter as well as IFN-β in a dose-dependent manner, whereas npmTRIMCyp was unable to induce NF-κB activation and showed a moderate effect on the stimulation of AP-1 and IFN-β. We also found the CypA domain of omTRIMCyp plays a critical role in NF-κB activation. The mechanism by which omTRIMCyp activates NF-κB is that omTRIMCyp facilitates induction of IκBα phosphorylation, however, which was not observed by npmTRIMCyp.

2. Materials and methods

2.1. Cells and plasmids

Human embryonic kidney (HEK) 293 T cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and incubated in a 5% CO_2 atmosphere at 37 ℃ with 100% humidity. The cells were passaged using standard cell culture techniques. Expression plasmids for hemagglutinin (HA)-tagged omTRIMCyp, npmTRIMCyp and chimeric TRIMCyp proteins (SWAP1 to SWAP5) were described previously (Kuang et al., 2009; Liu et al., 2015). pGL4.32 [luc2P/NF-κB-RE/Hygro] and pRL-TK vectors were purchased from Promega. AP-1 and IFN-β luciferase reporter plasmids were a kind gift from Dr. Hong-Bing Shu (Wuhan University, China). A Myc-tagged ubiquitin plasmid was a kind gift from Dr. Ce-Shi Chen (Kunming Institute of Zoology, Chinese Academy of Sciences).

2.2. Dual-luciferase assay

Transfection of HEK 293 T cells with different amount of plasmids (10–200 ng) expressing omTRIMCyp-HA, npmTRIMCyp-HA and empty vector LPCX-HA, together with plasmid DNA expressing firefly luciferase that is under the control of various transcriptional response element, including NF-κB (50 ng), AP-1 (100 ng) and IFN-β (100 and 200 ng), was conducted using Lipofectamine 2000 reagents (Invitrogen) as described by the manufacturer's instructions. In addition, a pRL-TK reporter plasmid was added to each transfection, which expresses *Renilla* luciferase for normalization of transfection efficiencies. Cells were harvested at 36 h post-transfection and luciferase activities were determined using a Dual-Luciferase Reporter Assay System (Promega) on a Flex Station 3 apparatus (Molecular Devices).

2.3. Quantitative real-time PCR

HEK 293 T cells were transfected with 200 ng of indicated TRIMCyps expression plasmids omTRIMCyp-HA and npmTRIMCyp-HA as well as empty vector LPCX-HA in a 12-well plate. Total RNA was extracted with TRIzol Reagent (Ambion) following the manufacturer's instructions. The concentration of RNA was quantified using a Nanodrop 2000 UV–vis Spectrophotometer (Thermo Fisher SCIENRIFIC), and cDNA was synthesized as previously described (Zhu et al., 2016). The resultant cDNA was then subjected to quantitative real-time PCR (qRT-PCR) using a SYBR Premix Ex TaqII (TliRNaseH Plus) Kit (TaKaRa), on a ViiA7 Real-Time PCR System apparatus (Applied Biosystems). PCR reactions were carried out with a holding stage of 30 s at 95 ℃ followed by 40 cycles of 3 s at 95 ℃, 60 ℃ for 30 s. Melt curves were performed after each PCR reaction to confirm the specificity of the amplification reactions.

The sequences of the primers used are as follows: GAPDH (M33197): 5'-GCTTCGCTCTCTGCTCCTCCTGTT-3'(F); 5'-ACGACCAAATCCGTTGACTCCGACC-3'(R), TAB1 (U49928) : 5'-CTGCCAAGTCCAAACCAATC-3'(F) ; 5'-GCAAACTCAGTGTCAATCATCG-3'(R), TAB2 (AF241230): 5'-GCTGAAAGACTTGGTTGCC-3'(F); 5'-CATTCTATTCCTTCCTCCTGTC-3'(R), TAB3 (AY371491): 5'-CGAACTGGCTCCACACAAAG-3'(F); 5'-GCATCTCGCACTGCTCACA-3'(R), TNF-α (NM_000594.3): 5'-GTGACAAGCCTGTAGCCCATGTT-3'(F); 5'-TTATCTCTCAGCTCCACGCCATT-3'(R), IL-6 (NM_000600.4): 5'-CGGGAACGAAAGAGAAGCTCTA-3'(F); 5'-GAGCAGCCCCAGGGAGAA-3'(R), IFN-β (M25460): 5'- CCAACAAGTGTCTCCTCCAAAT-3' (F); 5'-AATCTCCTCAGGGATGTCAAAG-3'(R), MxA (M30817.1): 5'-ACCTACAGCTGGCTCCTGAA-3'(F); 5'-GCACTCAAGTCGTCAGTCCA-3'(R), ISG15 (AY168648.1): 5'-AGCGAACTCATCTTTGCCAGTACA-3'(F); 5'-CAGCTCTGACACCGACATGGA-3'(R), c-Fos (NM_005252.3): 5'-GCATCTGAGAAGCCAAGACTGAGCC-3'(F); 5'-GAACATCATCGTGGCGGTTAGGC-3'(R), and c-Jun (NM_002228.3): 5'-TGAAACAGAGCATGACCCTGAACCT-3'(F); 5'-TGCCCGTTGCTGGACTGGATT-3'(R). The relative gene