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Original article

Inhibitory effect of human TRIM5α on HIV-1 production

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Abstract

Tripartite motif-containing 5 isoform-α (TRIM5α), a host restriction factor, blocks infection of some retroviruses at a post-entry, pre-integration stage in a species-specific manner. A recent report by Sakuma et al. describes a second antiretroviral activity of rhesus macaque TRIM5α, which blocks HIV-1 production through rapid degradation of HIV-1 Gag polyproteins. Here, we find that human TRIM5α limits HIV-1 production. Transient expression of TRIM5α decreased HIV-1 production, whereas knockdown of TRIM5α in human cells increased virion release. A single amino acid substitution (R437C) in the SPRY domain diminished the restriction effect. Moderate levels of human wild-type TRIM5α and a little amount of R437C mutant were incorporated into HIV-1 virions. The R437C mutant also lost restriction activity against N-tropic murine leukemia virus infection. However, the corresponding R to C mutation in rhesus macaque TRIM5α had no effect on the restriction ability. Our findings suggest human TRIM5α is an intrinsic immunity factor against HIV-1 infection. The importance of arginine at 437 aa in SPRY domain for the late restriction is species-specific.

Keywords: Human TRIM5α; HIV-1 production; Restriction factor; Intrinsic immunity; MLV

1. Introduction

The intrinsic host defense system has recently come to light as a key player in restricting retroviral infection. Several intrinsic factors important in limiting HIV-1 infection have been identified. APOBEC3G [1] and APOBEC3F [2] interfere with the replication of HIV-1 and other retroviruses via their cytidine deaminase activity [3,4]. The membrane protein Tetherin blocks the release of HIV-1 and other enveloped viruses by tethering them to the cellular membrane [5]. TRIM5α has been shown to be a post-entry restriction factor that confers resistance to HIV-1 in a species-specific manner. Even though these restriction factors are thought to be constitutively expressed and active before pathogen invasion [6,7], they are induced by interferon and therefore may constitute innate immune defenses [5,8,9].

TRIM5α has been proposed to bind the incoming viral capsid and interfere with uncoating [10,11]. It has also been reported that TRIM5α possesses E3 ubiquitin ligase activity and a mutation in its RING finger domain decreases its restriction ability [12–14]. Other reports suggest that TRIM5α prevents late RT product formation and inhibits viral cDNA nuclear import [15,16].

Recently, another antiretroviral activity of TRIM5α of rhesus monkey, TRIM5α_{rh}, has been described, which is inhibition of HIV-1 production at a post-translational stage by degradation of the Gag protein [17]. SIV is resistant to this restriction. However, another group argued against this effect of TRIM5α_{rh} [18] although both showed that overexpression of TRIM5α_{rh} down-regulates HIV-1 production.

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TRIM5α is a member of the tripartite motif protein family and contains RING, B-box 2, and coiled-coil domains, as well as a carboxy-terminal B30.2 (SPRY) domain. The contribution of each domain to HIV-1 post-entry restriction had been well documented. The RING domain contributes to the potency of restriction but is not absolutely essential. The B-box 2, coiled-coil and SPRY domains of TRIM5α are necessary for anti-retroviral activity. The coiled-coil domain is indispensable for TRIM5α oligomerization, which increases the avidity of TRIM5α for the retroviral capsid. Both the coiled-coil and SPRY domains are essential for TRIM5α to bind the virion core [19]. Variation in the SPRY domain is thought to be responsible for species-specific differences in retroviral restriction [19,20]. During the study for the late-restriction, the RING structure and B-box 2, coiled-coil and ensuing linker 2 domains are all found to be responsible, while the N-terminal region, RING and B-box2 domains have been shown to be essential for interaction between TRIM5α_{rh} and HIV-1 Gag. The coiled-coil domain and linker 2 region may play an effector function in late-restriction as well as the known effect on formation of the cytoplasmic body including TRIM5α_{rh} [21].

Human TRIM5α (TRIM5α_{hu}) had been considered to have no antiviral activity on HIV-1 production. However, during the course of our study comparing the TRIM5s of various species including primate TRIM5α and rodent TRIM5, we do found that TRIM5α_{hu} potently blocked the release of HIV-1 when expressed at the same level with TRIM5α_{rh}. Here, we provide the evidences that TRIM5α_{hu} inhibits production of HIV-1 progeny at physiological concentrations, suggesting it plays an important role in intrinsic defense against HIV-1.

2. Materials and methods

2.1. Cell culture

Human 293T, HeLa, HT1080, TZM-bl [22] and Plat-gp cells [23] were maintained in DMEM containing 10% FCS and antibiotics. Jurkat E6-1 cells (ATCC number: TIB-152) were cultured in RPMI1640 containing 10% FCS.

2.2. Plasmids

The plasmid pRhT5α, which encodes C-terminal hemagglutinin (HA)-tagged TRIM5α_{rh}, was provided by Dr. Ikeda. The plasmid encoding HA-tagged Trim5α_{hu} was obtained from Dr. Shioda, and designated pHuT5αWT. HA-tagged TRIM5α_{hu} cDNA harboring the R437C mutation was amplified by RT-PCR from human peripheral blood mononuclear cells (PBMC) with a previously described primer pair [17], and kindly provided by Dr. Y. Ikeda. It was then cloned into pcDNA3.1 (Invitrogen), the same vector that was used to generate pRhT5α and pHuT5αWT. The resultant plasmid was designated pHuT5αR437C. For construction of pRhT5αR441C, a corresponding mutant of TRIM5α_{hu}R437C, the plasmid pRhT5α was used as a template for QuikChange site-directed mutagenesis (Stratagene). To generate TRIM5α encoding retroviruses, the retroviral vector pMX-puro [24] and Plat-gp packaging cells were used. The HA-tagged TRIM5α open reading frames (ORFs) described above were amplified by PCR using primers huT5α-F-EcoRI (5′-GCGAATTCCACCATGGCTTCTGGAATCCTG-3′; Underline shows EcoRI site.) and NotI-HA-R (5′-AGATAA-GAATGCGGCCGCTCAAGCGTAATCTGGAACATCG-3′; Underline shows NotI site.) and then cloned into EcoRI and NotI sites of pMX-puro. The resulting plasmid was designated pMX-T5α-HA-puro. All of the TRIM5α proteins possess a carboxy-terminal HA tag to allow detection of the expression level. The HIV-1 molecular clone pNLΔpolEGFP was constructed by inserting the EGFP ORF in the Nef coding region of pNLΔpol [25] that lacks a 328 base pair fragment in the polymerase coding region of pNL4-3 [26]. The resultant plasmid produces the p55 Gag precursor protein that is processed to p24 capsid protein. The plasmid of pYK-JRCSF [27], pNL4-3, pSIVmac239 [28], pSA212, encoding full length genome of SIVagm [29] and p89.6 [30], were obtained from Dr. Y. Koyanagi, Dr. Adachi, Dr. Mori, Dr. Miura, and AIDS Research and Reference Reagent Program, respectively. The β-galactosidase (β-gal) expression plasmid pCDM-β-gal [31] was used.

2.3. Transfection and viral infection

Co-transfection of one of pNLΔpolEGFP, pYK-JRCSF, pNL4-3, p89.6, pSA212 and pSIVmac239 with TRIM5α expression plasmids (pRhT5α, pHuT5αWT, pHuT5αR437C) or empty vector pcDNA3.1 into 293T cells was carried out using Lipofectamine and Plus reagent (Invitrogen) according to the manufacturer's instructions. Two days later, the culture supernatants were harvested and the p24 concentration was measured with p24 Gag ELISA assay kit (Zeptmatrix). The p24 concentration was divided by intracellular β-gal activity evaluated by standard colorimetric methods. The value of control pcDNA3.1-transfected cells was set as 1, and compared with the value of various TRIM5α expression plasmid-transfected cells.

To measure the infectivity of progeny viruses, TZM-bl cells, the HeLa cell derivatives that express human CD4 and CCR5 and contain a luciferase construct under the control of the HIV-1 promoter, were seeded in 24-well plate (5×10^4/well). The next day, the medium was removed and the cells were incubated with 250 μl of HIV-1 or SIV harboring culture media for 3.5 h followed by adding 750 μl of fresh media. Forty-eight hours after infection, cells were washed and lysed, and then luciferase activity was measured using Promega's BrightGlo luciferase assay system. To calculate the production rate of infectious virus, the ratio of luciferase activities against the β-gal activities in HIV-1/SIV producing 293T cell lysates was calculated and the value of control pcDNA3.1-transfected sample was set as 1.

2.4. Preparation of viral like particle (VLP)

The culture supernatants (2 ml) were harvested 48 h post-transfection, and cellular debris was removed by centrifugation