Short Communication

MCPIP1/regnase-I inhibits simian immunodeficiency virus and is not counteracted by Vpx

Hongmei Li¹ and Tony T. Wang²

¹Research Institute of Medicine and Pharmacy, Qiqihar Medical University, 333 Bukui North Street, Heilongjiang 161006, PR China
²Center for Infectious Diseases, Bioscience Division, SRI International, 140 Research Drive, Harrisonburg, VA 22802, USA

We have previously shown that the cellular RNase MCPIP1/regnase-1 potently blocks HIV-1 infection in resting CD4⁺ T-cells. As simian immunodeficiency virus (SIV) encodes an accessory protein named Vpx, which enhances viral replication in resting CD4⁺ T-cells by degrading the cellular restriction factor SAMHD1, we investigated whether MCPIP1 restricts SIV infection and whether Vpx protein antagonizes MCPIP1-mediated restriction. In co-transfection studies, human MCPIP1 markedly reduced the production of infectious SIV, whereas MCPIP2 and MCPIP3 had little effect. MCPIP1 derived from cynomolgus monkey also inhibited human immunodeficiency virus (HIV-1) and SIV production, albeit to a lesser degree. Lastly, expression of SIV Vpx protein did not reduce MCPIP1 at the protein level, nor did it ablate the MCPIP1-mediated restriction. In conclusion, both human and cynomolgus monkey MCPIP1 restrict SIV replication. Unlike SAMHD1, MCPIP1-mediated HIV-1 restriction cannot be overcome by SIV Vpx.

Received 21 March 2016
Accepted 12 April 2016

Human immunodeficiency virus (HIV-1) preferentially infects activated cluster of differentiation (CD4⁺) T-cells and macrophages over resting CD4⁺ T-cells. Extensive research effort in the past decade has now revealed multiple proteins in those refractory cell types that are able to put the brakes on almost every step of the viral life cycle. For example, the apolipoprotein B mRNA-editing enzyme 3G (APOBEC3G) and related proteins are cytidine deaminases that mutate the viral DNA and render virus progeny less infectious (Hache et al., 2005; Harris et al., 2003; Harris & Liddament, 2004; Mangeat et al., 2003; Sheehy et al., 2002; Zhang et al., 2003); rhesus TRIM5α disrupts the viral capsid soon after entry and hence stops infection prior to reverse transcription (Stremlau et al., 2004); tetherin prevents the virosome from budding off (Neil et al., 2008); the interferon-inducible factor, MX2/MxB, inhibits HIV infection by inhibiting capsid-dependent nuclear import or integration (Kane et al., 2013; Liu et al., 2013b); and BCA2/Rabring7 targets HIV-1 Gag for lysosomal degradation in a tetherin-independent manner (Nityanandam & Serra-Moreno, 2014). Most recently, the cellular sterile alpha motif (SAM) domain HD domain protein 1 (SAMHD1) was found to decrease the cellular pool of nucleotide to restrict HIV-1 infection of dendritic and myeloid cells (Berger et al., 2011; Goldstone et al., 2011; Hrecka et al., 2011; Laguette et al., 2011; Lahouassa et al., 2012). Notably, SAMHD1 is also known to restrict HIV-1 infection in resting CD4⁺ T-cells (Baldauf et al., 2012; Descours et al., 2012), and its effect can be overcome by the HIV-2 or SIV-encoded accessory protein Vpx (Berger et al., 2011; Hrecka et al., 2011; Laguette et al., 2011), which targets SAMHD1 for cullin4A-RING E3 ubiquitin ligase (CRL4)-dependent degradation (Ahn et al., 2012; Bergamaschi et al., 2009).

We and others have reported that the CCCH zinc-finger protein MCPIP1 drastically reduced the steady levels of multiple HIV-1 RNA species without directly inhibiting the HIV-1 LTR transcription (Liu et al., 2013a). We and others also found that MCPIP1 inhibits a broad spectrum of viruses (Lin et al., 2013; Liu et al., 2013a). MCPIP1 belongs to a novel family of four proteins whose expression is highly enriched in immune tissues. It was previously recognized as a critical regulator of the inflammatory response and immune homeostasis (Liang et al., 2008, 2010; Matsushita et al., 2009). MCPIP1 acts as an RNase to promote the mRNA degradation of some inflammatory cytokines (Iwasaki et al., 2011; Matsushita et al., 2009) and cleave precursor microRNAs (pre-miRNAs) (Suzuki et al., 2011). Consistent with this, mutations that abolish the RNase

Supplementary methods and two supplementary figures are available with the online Supplementary Material.

000482 © 2016 The Authors Printed in Great Britain

1693
Fig. 1. MCPIP1 restricts SIV infection. To package SIV-GFP virus carrying VSV glycoprotein (VSV-G), 2 µg pShvec1.GFP, 0.2 µg VSV-G and 0.1 µg Rev plasmid were transfected into 293T cells along with 0.5 µg hMCPIP1 or the plasmids indicated. For HIV-GFP virus, 0.5 µg pTrip-GFP, 0.5 µg pCMVR8.2 and 0.2 µg VSV-G were co-transfected with 0.5 µg of the MCPIP1 plasmids indicated; for MLV-GFP virus, 0.25 µg pFB-GFP, 0.2 µg MLV-Gag-pol and 0.1 µg VSV-G were co-transfected with 0.25 µg MCPIP1 plasmid. Forty-eight hours after transfection, cells were imaged by Nikon TS-100 fluorescence microscope. 525 or 125 µl supernatants were added to native 293T cells for virus titration. Infected cells were both imaged and quantified by flow cytometry for GFP+ cells in order to calculate infectious titre [infectious units (i.u.) ml⁻¹]; n=3.
activity of MCPIP1 also ablated its ability to restrict HIV-1 virus infection (Liu et al., 2013a).

Previously it was shown that SIV Vpx degrades endogenous SAMHD1, enabling early steps of the HIV-1 life cycle (Baldauf et al., 2012; Descours et al., 2012). Bypassing SAMHD1 in resting CD4\(^+\) T-cells by Vpx treatment, however, was insufficient for the release of viral progeny, implicating other barriers at later stages of HIV replication (Baldauf et al., 2012). To determine if MCPIP1 represents one of such barriers that acts at a later stage, we sought to determine whether MCPIP1 restricts SIV infection and, moreover, whether MCPIP1-mediated restriction is antagonized by SIV-encoded Vpx. To this end, we first carried out co-transfection experiments (see Supplementary Methods, available in the online Supplementary Material), in which DNA plasmid expressing human MCPIP1 (hMCPIP1) was co-transfected with a SIV-GFP plasmid, a SIV-packaging plasmid, and vesicular stomatitis virus (VSV) glycoprotein (VSV-G). We monitored the transfection efficiency, i.e. the expression of SIV-GFP in transfected cells, and subsequently collected the supernatants and titred the production of infectious virus on naïve 293T cells. As shown in Fig. 1, MCPIP1 expression did not affect the transfection efficiency as transfected cells showed similar levels of GFP expression. By contrast, the amount of infectious SIV-GFP and HIV-GFP in the supernatants decreased by more than two orders of magnitude in the presence of MCPIP1 expression. The amount of the infectious MLV-GFP was not altered much even in the presence of MCPIP1, which is consistent with our previous observation (Liu et al., 2013a). Next, we cloned cynomolgus monkey MCPIP1 (MonMCPIP1) from relevant RNA samples (obtained from Dr Cristian Apetrei, University of Pittsburgh, USA). When tested, MonMCPIP1 also reduced SIV and HIV-1 production (Fig. 1). This is not surprising given the 97% sequence identity between hMCPIP1 and MonMCPIP1 (Fig. S1). Of note, the protein sequence of rhesus macaque MCPIP1 (NCBI XP_014991141) is identical to that of MonMCPIP1.

To probe the ability of Vpx to counteract MCPIP1, we transfected 293T cells with Myc-tagged SIVmac239 Vpx plasmid along with HA-SAMHD1 or Flag-MCPIP1. Increasing the amount of Vpx significantly decreased SAMHD1, but had no effect on MCPIP1 expression (Fig. 2a). In support, MCPIP1-dependent restriction of HIV-1 was not reversed in the presence of Vpx (Fig. 2b). In support, MCPIP1 primarily localized to the cytoplasm whereas Vpx and SAMHD1 were predominantly held in the nucleus (Fig. 3a–d). Altogether, our data suggest that MCPIP1-mediated restriction cannot be overcome by SIV Vpx. By contrast, MCPIP1 is known to be cleaved by mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1) and degraded in activated CD4\(^+\) T-cells (Jeltsch et al., 2014; Liu et al., 2013a; Uehata et al., 2013). It is highly likely that MCPIP1 may contribute to the resistance observed by resting CD4\(^+\) T-cells to HIV-1 infection, and MCPIP1-mediated restriction is removed upon cellular activation. In support, we recently reported that blocking MALT1 enzymic activity by a small molecule named MI-2 induced MCPIP1 protein levels in human T-cells and increased resistance to HIV (Li et al., 2016).

![Fig. 2. SIV Vpx does not antagonize MCPIP1.](image-url)
MCPIP1 is a CCCH-type zinc-finger protein that harbours RNase activity (Matsushita et al., 2009; Suzuki et al., 2011). Mutations that are known to abolish its RNase activity (D141N) and the zinc-finger domain (C306R) both ablated the ability of MCPIP1 to restrict HIV and SIV (Liu et al., 2013a) (Fig. 1). We previously reported that MCPIP1 reduced the abundance of viral mRNA species without affecting the HIV LTR-dependent transcription (Liu et al., 2013a). In that study, however, only the basal LTR promoter activity was measured. To extend the finding, we assessed how induction of MCPIP1 affects HIV LTR-driven promoter activity when stimulated by the protein kinase C agonist bryostatin 1 or by HIV Tat protein. Using doxycycline-inducible MCPIP1 clones derived from a HeLa cell line, we demonstrated that both bryostatin-1- and Tat-induced HIV transcription were modestly downregulated by MCPIP1 (Fig. S2a, b). In contrast, in CEM-derived doxycycline-inducible MCPIP1 clones, induction of MCPIP1 had very little effect on bryostatin-1- or Tat-induced HIV transcription (Fig. S2c, d). Taken together, either the current HIV LTR promoter region is not targeted by MCPIP1 for degradation or MCPIP1 does not affect HIV transcription at all because LTR-dependent transcription is

**Fig. 3.** MCPIP1 localizes to cytoplasm. (a) Two distribution patterns of GFP-hMCPIP1 were observed in HEK293-Dox-GFP-MCPIP1 cells after doxycycline induction. Bars, 10 µm (cytoplasmic), 5 µm (punctate). (b) Flag-hMCPIP1 was immunostained using the Millipore mab-MCPIP1 (2H8.2, 1:100) in HeLa-Dox-Flag-hMCPIP1 cells after induction. A representative image is presented of cells expressing low amounts of Flag-MCPIP1. Bars, 5 µm (control), 10 µm (Flag-MCPIP1). (c–e) 293T cells were transfected with the plasmids indicated and fixed in methanol prior to immunostaining. Images were captured using Carl Zeiss LSM 700 confocal microscopy. The doxycycline-inducible clones were previously established in the lab (Liu et al., 2013a). Bars, 5 µm.
readily induced by stimuli even in the presence of MCPIP1. Alternatively, MCPIP1 may inhibit HIV transcription in a cell-type-specific manner. Regardless, it would be interesting to determine the exact region of HIV mRNA species that may be targeted by MCPIP1 for degradation.

In conclusion, MCPIP1 appears to impose its restriction via an entirely different mechanism from SAMHD1. It does not co-localize with SAMHD1, nor can it be overcome by Vpx. Instead MCPIP1-mediated restriction in resting CD4+ T-cells is relieved upon cellular activation which promotes rapid degradation of MCPIP1 (Liu et al., 2013a; Uehata et al., 2013). MCPIP1 does appear to partially co-localize with APOBEC3G (Fig. 3e), although this finding needs further confirmation in immune-relevant cells at endogenous levels. Notably, APOBEC3G reportedly localizes to mRNA processing (P) bodies and stress granules (Gallois-Montbrun et al., 2007; Wichroski et al., 2006), cytoplasmic compartments involved in the degradation and storage of nontranslating mRNAs. Whether APOBEC3G and MCPIP1 assemble into a ribonucleoprotein complex warrants future investigation as this will likely provide novel insights into understanding how these factors restrict HIV infection. Nevertheless, the post-transcriptional inhibition exerted by MCPIP1 may pose another barrier for resting CD4+ T-cells to release progeny even when SAMHD1 is removed. Further insight into the regulation and consequences of the MCPIP1-imposed restriction in CD4+ T-cells may help identify new pathways for interfering with immunodeficiency in HIV-1-infected individuals.

Acknowledgements

The authors are grateful to Drs Skowronski, Landau, Sodroski and Cosset for providing DNA plasmids. This study was sponsored by National Institute of Health grant R01DK088787 (to T.W.) and by Natural Sciences and Engineering Research Program of Heilongjiang Education Department of China for providing DNA plasmids. This study was sponsored by National Science Foundation of Heilongjiang Province grants QC2012C094 (to H. L.). H. L. is a recipient of the ‘Reserve Talents of Universities Overseas Research Program of Heilongjiang Education Department’. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

References


Haché, G., Liddament, M. T. & Harris, R. S. (2005). The retroviral hypermutation specificity of APOBEC3F and APOBEC3G is governed by the C-terminal DNA cytosine deaminase domain. <i>J Biol Chem</i> 280, 10920–10924.


Iwasaki, H., Takeuchi, O., Teraguchi, S., Matsushita, K., Uehata, T., Kuniyoshi, K., Satoh, T., Saitoh, T., Matsushita, M. & other authors (2011). The IeB kinase complex regulates the stability of cytokine-encoding mRNA induced by TLR-IL-1R by controlling degradation of roquin and regnase-1. <i>Nat Immunol</i> 12, 1167–1175.


