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Functional interplay between the B-box 2 and the B30.2(SPRY) domains of TRIM5α

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Abstract

The retroviral restriction factors, TRIM5α and TRIMCyp, consist of RING and B-box 2 domains separated by a coiled coil from carboxy-terminal domains. These carboxy-terminal domains (the B30.2(SPRY) domain in TRIM5α and the cyclophilin A domain in TRIMCyp) recognize the retroviral capsid. Here we show that some B-box 2 changes in TRIM5α, but not in TRIMCyp, resulted in decreased human immunodeficiency virus (HIV-1) capsid binding. The phenotypic effects of these B-box 2 changes on the restriction of retroviral infection depended on the potency of restriction and the affinity of the TRIM5α interaction with the viral capsid, two properties specified by the B30.2(SPRY) domain. Thus, some alterations in the TRIM5α B-box 2 domain apparently affect the orientation or conformation of the B30.2(SPRY) domain, influencing capsid recognition.

Keywords: Retrovirus; Restriction factor; TRIMCyp; Cyclophilin; Trimer; B-box 2; B30.2(SPRY); HIV-1; N-MLV

Introduction

Retroviruses encounter potent blocks to infection in certain mammalian species. In many cases, the restriction is mediated by dominant host factors, such as Fv1 (Best et al., 1996; Hartley et al., 1970; Lilly, 1967; Lilly, 1970), APOBEC3G (Sheehy et al., 2002) and TRIM5α (Stremlau et al., 2004). TRIM5α blocks retroviral infection at an early post-entry step in a species-specific manner. Rhesus monkey TRIM5α ($TRIM5\alpha_{rh}$) potently blocks the infection of human immunodeficiency virus-1 (HIV-1) and a range of other retroviruses; by contrast, human TRIM5α ($TRIM5\alpha_{hu}$) modestly restricts HIV-1 infection and potently blocks infection by N-tropic murine leukemia viruses (N-MLV) (Hatziioannou et al., 2004; Keckesova et al., 2004; Perron et al., 2004; Stremlau et al., 2004; Yap et al., 2004). The mechanism by which TRIM5α blocks retroviral infection has not been clearly defined. Previous studies demonstrated that TRIM5α proteins specifically recognize the viral cores and accelerate the uncoating process (Li et al., 2006b; Perron et al., 2007; Sebastian and Luban, 2005; Stremlau et al., 2006), thus potentially interfering with the orderly disassembly of the viral capsid (Forshey et al., 2002).

TRIM5α is a member of the large tripartite motif (TRIM) protein family (Reymond et al., 2001). TRIM proteins contain RING, B-box 2 and coiled-coil domains; many cytoplasmic TRIM proteins, like TRIM5α, also contain a B30.2(SPRY) domain (Meroni and Diez-Roux, 2005; Reymond et al., 2001). The B30.2(SPRY) domain of TRIM5α determines viral specificity and potency of restriction by modulating recognition of the retroviral capsid (Stremlau et al., 2005; Yap et al., 2005; Perez-Caballero et al., 2005a; Li et al., 2006b). For example, in an *in vitro* binding assay, the B30.2(SPRY) domain was shown to be the major determinant of the association of $TRIM5\alpha_{rh}$ with the assembled HIV-1 capsid-nucleocapsid (CA-NC) complex (Li et al., 2006b; Stremlau et al., 2006).

The role of TRIM5α domains other than the B30.2(SPRY) domain in antiretroviral activity has been investigated. The RING finger domain is the signature of a class of E3 ubiquitin ligases involved in proteasome-mediated protein degradation (Meroni and Diez-Roux, 2005). Indeed, some TRIM proteins

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have been shown to exhibit ubiquitin ligase activity and modify their target proteins with ubiquitin (Dupont et al., 2005; Horn et al., 2004; Meroni and Diez-Roux, 2005; Trockenbacher et al., 2001; Xu et al., 2003). However, deletion of the TRIM5α RING domain only partially attenuated antiviral activity (Javanbakht et al., 2005; Perez-Caballero et al., 2005a; Stremlau et al., 2004). Moreover, TRIM5α-mediated restriction activity was not affected by modulation of E1 ubiquitin ligase activity in a temperature-dependent cell line (Perez-Caballero et al., 2005b). Finally, proteasome inhibitors did not impair the restriction activity of TRIM5α (Perez-Caballero et al., 2005b; Stremlau et al., 2006; Anderson et al., 2006; Wu et al., 2006). Proteasome inhibitors can rescue viral reverse transcription from the TRIM5α-mediated restriction, but the mechanistic basis for this phenomenon is unknown (Anderson et al., 2006; Wu et al., 2006). The B-box 2 domain plays an important but poorly understood role in the function of TRIM proteins (Meroni and Diez-Roux, 2005; Reymond et al., 2001). Deletion of the B-box 2 domain of TRIM5α eliminated its antiretroviral activities (Javanbakht et al., 2005; Perez-Caballero et al., 2005a). It has been suggested that the RING and B-box 2 domains may specify an "effector" function, either by recruiting an additional co-factor or by participating in particular types of self-association (Diaz-Griffero et al., 2006b; Perez-Caballero et al., 2005a; Stremlau et al., 2006). The coiled-coil domain is known to be essential for both homomultimerization and heteromultimerization of many TRIM proteins (Cao et al., 1997; Javanbakht et al., 2006; Mische et al., 2005; Perez-Caballero et al., 2005a; Reymond et al., 2001). TRIM5α trimerization, which depends on the coiled coil and the L2 linker region connecting the coiled-coil and B30.2 domains, contributes greatly to the avidity for the retroviral capsid and the restriction of viral infection (Javanbakht et al., 2006).

In this study, we altered a combination of residues in the B-box 2 domain of wild-type human and rhesus monkey TRIM5α, as well as chimeric and mutant TRIM5α proteins, and examined the effects of these changes on antiretroviral activity against human immunodeficiency virus-1 (HIV-1), N-tropic Moloney leukemia virus (N-MLV) and simian immunodeficiency virus (SIV_{mac}). The results show that, depending on the restriction potency of the parental proteins, a property determined by the B30.2(SPRY) domain, the same alterations of the B-box 2 domain can confer different phenotypes on retrovirus restriction. Moreover, the B-box 2 alterations used in this study dramatically affected the association of the TRIM5α proteins with *in vitro* assembled HIV-1 CA-NC complexes, suggesting a functional interplay between the TRIM5α B-box 2 and the B30.2(SPRY) domains.

Results

Differential effects of TRIM5α B-box 2 changes on HIV-1 and N-MLV restriction

Despite 87% sequence identity, rhesus monkey TRIM5α ($TRIM5\alpha_{rh}$) and human TRIM5α ($TRIM5\alpha_{hu}$) exhibit differences in the spectrum of restricted retroviruses due to divergence in the B30.2 domains (Perez-Caballero et al., 2005a; Perron et al., 2006; Stremlau et al., 2005; Yap et al., 2005). $TRIM5\alpha_{rh}$ more potently restricts HIV-1 infection than $TRIM5\alpha_{hu}$, whereas $TRIM5\alpha_{hu}$ more potently restricts the infection of N-MLV (Hatziioannou et al., 2004; Keckesova et al., 2004; Perron et al., 2004; Stremlau et al., 2004; Yap et al., 2004). In a previous study, we generated two B-box 2 mutants of $TRIM5\alpha_{rh}$, each with a few amino acid residues changed to that seen in a relatively distant TRIM protein relative, TRIM21 (Li et al., 2006a). The TRIM21 B-box 2 domain, in contrast to B-box 2 domains of close TRIM5 relatives like TRIM6 or TRIM34, does not effectively substitute for that of $TRIM5\alpha_{rh}$ (Li et al., 2006a). Mutant Cluster I-A exhibits the following changes: Q109E, E110K, V114A, I115L and L118V; Mutant Cluster II-A exhibits the following changes: E120A, R121Q, Q123R and E124K (Fig. 1). The wild-type and mutant $TRIM5\alpha_{rh}$ proteins were expressed in HeLa cells (Fig. 2A). Both B-box 2 mutants restricted HIV-1 infection nearly as efficiently as the wild-type $TRIM5\alpha_{rh}$ protein (Fig. 2B). However, no anti-N-MLV activity was detected for either of the mutants. In fact, N-MLV infected cells expressing the Cluster I-A and Cluster II-A $TRIM5\alpha_{rh}$ mutants more efficiently than cells transduced with the control LPCX vector; these mutants may exert dominant-negative effects on the $TRIM5\alpha_{hu}$ protein endogenously expressed in the HeLa cells. Thus, the B-box 2 changes confer different phenotypes with respect to the anti-HIV-1 and the anti-N-MLV activities of rhesus monkey TRIM5α.

To investigate the phenotypes of the B-box 2 mutants in the context of more potent restriction against N-MLV infection, the same changes were introduced into $TRIM5\alpha_{hu}$. Interestingly, both $TRIM5\alpha_{hu}$ mutants retained anti-N-MLV activity, but lost the weak restricting activity against HIV-1 associated with wild-type $TRIM5\alpha_{hu}$ (Fig. 2B). These results demonstrate that changes in the B-box 2 domain of TRIM5α can affect both HIV-1 and N-MLV restriction. Apparently, if the parental TRIM5α is a potent restrictor of a specific virus, then the restriction activity directed against that virus can better tolerate the changes introduced into the B-box 2 domain.

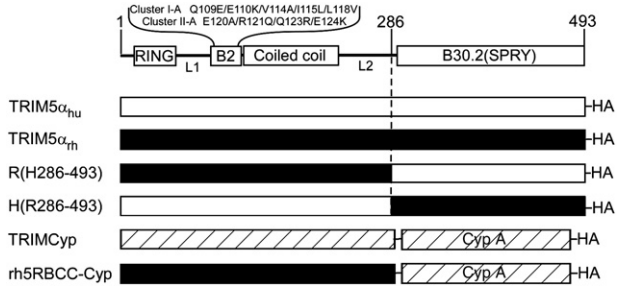

Fig. 1. TRIM5 and TRIMCyp constructs. The human and rhesus monkey TRIM5α proteins, $TRIM5\alpha_{hu}$ and $TRIM5\alpha_{rh}$ chimeric proteins, and the owl monkey TRIMCyp protein are depicted. A chimeric rh5RBCC-Cyp protein in which the amino-terminal portion of $TRIM5\alpha_{rh}$ was fused with the cyclophilin A domain of owl monkey TRIMCyp was also studied. The changes introduced into the B-box 2 domain of the proteins to create the Cluster I-A and II-A mutants are shown. The carboxy-terminal HA tag is shown.