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Effects of human *TRIM5α* polymorphisms on antiretroviral function and susceptibility to human immunodeficiency virus infection

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Abstract

TRIM5α acts on several retroviruses, including human immunodeficiency virus (HIV-1), to restrict cross-species transmission. Using natural history cohorts and tissue culture systems, we examined the effect of polymorphism in human TRIM5α on HIV-1 infection. In African Americans, the frequencies of two non-coding SNP variant alleles in exon 1 and intron 1 of *TRIM5* were elevated in HIV-1-infected persons compared with uninfected subjects. By contrast, the frequency of the variant allele encoding TRIM5α 136Q was relatively elevated in uninfected individuals, suggesting a possible protective effect. TRIM5α 136Q protein exhibited slightly better anti-HIV-1 activity in tissue culture than the TRIM5α R136 protein. The 43Y variant of TRIM5α was less efficient than the H43 variant at restricting HIV-1 and murine leukemia virus infections in cultured cells. The ancestral *TRIM5* haplotype specifying no observed variant alleles appeared to be protective against infection, and the corresponding wild-type protein partially restricted HIV-1 replication *in vitro*. A single logistic regression model with a permutation test indicated the global corrected P value of <0.05 for both SNPs and haplotypes. Thus, polymorphism in human *TRIM5* may influence susceptibility to HIV-1 infection, a possibility that merits additional evaluation in independent cohorts.

Keywords: TRIM5α; Single nucleotide polymorphism; HIV-1; HIV infections; Disease susceptibility; Genetics; Haplotype

Introduction

A major barrier to cross-species transmission of retroviruses is mediated by the TRIM5α protein (Bieniasz, 2003; Stoye, 2002; Stremlau et al., 2004). Variants of TRIM5α in different primate species block the early, post-entry phase of infection of cells by particular retroviruses (Hatziioannou et al., 2004b; Keckesova et al., 2004; Perron et al., 2004; Yap et al., 2004b). For example, even when expressed at comparable levels, human TRIM5α ($TRIM5\alpha_{hu}$) is less potent at suppressing infection of human immunodeficiency virus (HIV-1) than the rhesus monkey ortholog, $TRIM5\alpha_{rh}$ (Hatziioannou et al., 2004b; Keckesova et al., 2004; Stremlau et al., 2004; Yap et al., 2004a). On the other hand, $TRIM5\alpha_{hu}$ more potently restricts infection by the N-

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tropic murine leukemia virus (N-MLV) than TRIM5α_{rh} (Hatziioannou et al., 2004b; Perez-Caballero et al., 2005; Perron et al., 2004; Song et al., 2005c; Stremlau et al., 2004; Yap et al., 2004a).

TRIM5 is a member of a family of proteins that contain a *tri*partite *m*otif, hence the designation TRIM (Reymond et al., 2001). TRIM proteins have also been called RBCC proteins because the tripartite motif includes a *R*ING domain, *B*-box 2 domain and *c*oiled *c*oil domain. TRIM proteins exhibit the propensity to assemble into cytoplasmic or nuclear bodies (Reymond et al., 2001). Many cytoplasmic TRIM proteins contain a C-terminal B30.2 or SPRY domain. Differential splicing of the *TRIM5* primary transcript gives rise to the expression of several isoforms of the protein product. The TRIM5α isoform is the largest product (~493 amino acid residues in humans) and contains the B30.2(SPRY) domain. The B30.2(SPRY) domain of rhesus monkey TRIM5α is essential for anti-HIV-1 activity (Stremlau et al., 2005). Moreover, the difference in the anti-HIV-1 potency of rhesus and human TRIM5α proteins is determined by B30.2(SPRY) sequences (Perez-Caballero et al., 2005; Stremlau et al., 2005; Yap et al., 2005). An intact RING domain also contributes, either directly or indirectly, to the antiretroviral activity of TRIM5α_{rh} and TRIM5α_{hu} (Javanbakht et al., 2005; Perez-Caballero et al., 2005; Stremlau et al., 2005). The B-box 2 domain appears to be essential for efficient retrovirus restriction (Javanbakht et al., 2005; Perez-Caballero et al., 2005).

Interspecies differences in primate TRIM5α proteins dictate the potency of restriction against particular retroviruses (Hatziioannou et al., 2004b; Keckesova et al., 2004; Song et al., 2005c; Stremlau et al., 2004; Yap et al., 2004a). The effect of intraspecies variation in TRIM5α on antiretroviral potency is unknown. Moreover, it is uncertain whether the modest inhibitory effect of TRIM5α_{hu} on HIV-1 infection in tissue-cultured cells translates into any limiting effect on HIV-1 infection of humans. Functional polymorphisms in human *TRIM5* that correlate with differential susceptibility to HIV-1 infection or disease progression would support an interaction of TRIM5α_{hu} with HIV-1 *in vivo*. We therefore studied the influence of human *TRIM5* polymorphisms on HIV-1 infection and progression to AIDS in five U.S.-based natural history cohorts, an approach that has identified several other AIDS-modifying host variants (An et al., 2004; Dean et al., 1996; O'Brien et al., 2000). We also investigated the antiretroviral activity in tissue-cultured cells of common and several rare *TRIM5*α_{hu} variants.

Results

Identification of human TRIM5 single nucleotide polymorphisms (SNPs)

Common and rare *TRIM5* SNPs were identified in the human *TRIM5* gene using the SNP discovery panel (n=188), representing the extremes of the distribution for HIV-1 progression and infection, and from 359 normal blood donors and CEPH family founders, the Human Diversity Panel (n=368), at-risk HIV-1-uninfected individuals (n=344), and HIV-1-infected persons from the 5 U.S.-based natural history cohorts (n=698) and from South African HIV-1-infected and -uninfected Xhosa (n=272). The SNP discovery panel of 188 DNA samples was re-sequenced across all exons and intron–exon boundaries, and the other groups were re-sequenced for exon 2 encoding the RING domain and exon 8 encoding the B30.2(SPRY) domain. In addition, DNAs from persons progressing to AIDS in 5 or less years were re-sequenced for coding exons 2, 3, 8 and intron 6 and at-risk HIV-1-uninfected individuals were re-sequenced for exons 2, 3 and 8.

Twelve *TRIM5* SNPs having allele frequencies of >5% in either European Americans (EA) or African Americans (AA) were identified either by re-sequencing and/or from the NCBI dbSNP database; these common SNPs are listed in Table 1 and their locations indicated in Fig. 1. Of the 6 open reading frame (ORF) SNPs, 5 were non-synonymous and one was synonymous. For SNP4, SNP9 and SNP11, the minor alleles in AA were the major alleles in EA. These three SNPs also had the highest F_{st} (a measure of population differentiation) ranging from 0.23 to 0.35 (Supplementary Table 1). The remaining SNPs had F_{st} values ranging from 0.002 to 0.16 compared to an average F_{st} of 0.12 across the autosomes (Altshuler et al., 2005).

We also re-sequenced a large number of subjects to identify variant alleles in other populations (human diversity panel and the South African Zhosa) as well as participants enrolled in the AIDS cohorts with unusual patterns of progression (unusually rapid or slow progression). Nearly all of these additional variant alleles were infrequent and are listed in Table 2. In the Zhosa, only one variant allele had a frequency in the 4–5% range. Two non-synonymous SNPs were observed in only the Xhosa population. P479L was polymorphic in both HIV-1-infected (minor allele frequency (MAF) ≈5%) and -uninfected Xhosa (MAF ≈4%) but was observed only once in a European American. Because these SNPs are rare, their possible effects on infection or progression could not be assessed: SNP distribution between HIV-1 seronegatives and seropositives are listed in Table 2.

Selection on TRIM5

Perturbations in the allele frequency spectrum can reflect relatively recent selective episodes. The SNP discovery panel (unambiguous DNA sequences for all exons were available for 155 subjects) suggests a scarcity of low frequency polymorphism (range 1–5%) *TRIM5*α SNP alleles (6.5% for H43Y, 6.1% for Y112F, 26.8% for R136Q, 20% for L159L, 12.9% for G249D and 1.9% for H419Y) throughout the 1.5-kb protein-coding segment. However, the large screen (Table 2) contained a number of rare variant alleles at frequencies less than 1%. The allele spectrum does not deviate significantly from neutrality—Tajima's D=−1.306 (P>0.1).

Past or ongoing selection might also be revealed in the ratio of synonymous to non-synonymous changes. In humans, only 5 of 24 coding region variants are synonymous (Tables 1 and 3) and only 2 of 17 substitutions found between human and