Published in final edited form as:

AIDS. 2013 February 20; 27(4): . doi:10.1097/QAD.0b013e32835af34c.

Polymorphic LEDGF/p75 variants support efficient HIV-1 infection ex vivo

Yasuhiro Koh^a, Ester Ballana^b, José Este^b, and Alan Engelman^a

^aDepartment of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA

^bIrsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badolona, Barcelona, Spain

HIV-1 replication relies on interactions with numerous cellular factors [1], and genetic polymorphisms in the human population can significantly effect progression to AIDS [2, 3]. Lens epithelium-derived growth factor (LEDGF)/p75 is a crucial co-factor that directs HIV-1 integration to active genes [4]. N-terminal elements that include a PWWP domain confer constitutive binding to chromatin, whereas the downstream IN-binding domain (IBD; residues 347–429) binds lentiviral IN protein [5, 6]. IBD or PWWP domain point mutations can render LEDGF/p75 nonfunctional in ex vivo models of HIV-1 infection [7–9].

A recent study identified polymorphisms in *PSIP1*, the gene that encodes LEDGF/p75, which trended with reduced viral loads among Black South Africans [10]. Most of these were intronic though one mutation, Q472L, mapped nearby the IBD (Fig. 1a). A subsequent study identified two mutations in the same region, I436S and T473I, among Caucasoid Spanish long-term nonprogressors, though the relatively small size of the study group precluded disease association analysis [11]. The Q472L mutation did not affect IN binding in vitro or co-factor function in cells depleted for LEDGF/p75 content by RNA interference (RNAi) [10]. Initial RNAi-based studies notably failed to reveal a role for LEDGF/p75 in HIV-1 infection despite achieving potent reductions in steady-state protein levels [12]. Subsequent “deep knockdown” [7] and *Psip1* knockout [8, 13] studies revealed approximate 10 to 30-fold reductions in viral titer, indicating that residual levels of LEDGF/p75 can support efficient HIV-1 infection [7, 14]. We have therefore tested the affects of the I436S, Q472L, and T473I mutations on HIV-1 infection using cells derived from *Psip1* knockout mice that lack the ability to synthesize LEDGF/p75 [8].

Two different mouse embryo fibroblast (MEF) cell lines, E6(–/–) and E2(–/–) [8, 9], were utilized to assess generalized responses. The pIRES2-eGFP-LEDGF-HA vector that co-expresses human LEDGF/p75 fused to a hemagglutinin (HA) tag and green fluorescent protein (GFP) was mutagenized by PCR, and resulting plasmids were verified by sequencing [8]. Transfected cells (4×10^6) were plated for 24 h prior to fluorescent-activated cell sorting to select GFP-positive cells. Sorted cells (2×10^4) were infected with HIV-Luc, a single-round reporter virus that carries a deletion in the *env* gene and the gene for firefly luciferase in *nef*; HIV-Luc was pseudotyped with the vesicular stomatitis virus G glycoprotein to afford entry, reverse transcription, and integration in mouse cells [8]. Cells infected in duplicate were lysed after 48 h, and resulting levels of luciferase activity, expressed as relative light units (RLU), was normalized to total protein as determined by Bradford assay (Bio-Rad).

Conflicts of interest There are no conflicts of interest.

As expected, E2(−/−) cells expressing wild-type (WT) LEDGF/p75 supported about 10-fold greater levels of HIV-Luc infection than cells transfected with the empty pIRES2-eGFP vector [8, 9] (Fig. 1b). Cells transfected with each of the mutant LEDGF/p75 vectors supported infection at levels that were indistinguishable from WT LEDGF/p75; subtracting baseline LEDGF/p75-independent values and re-plotting mutant activity as percent WT function revealed marginally lower activity for the Q472L variant (Fig. 1c) that failed to attain statistical significance as determined by one-tailed Student's *t*-test ($P = 0.32$). Asp366 in the IBD makes critical contacts with the IN polypeptide backbone [15], and E6(−/−) cells were additionally transfected with the LEDGF/p75 mutant D366N vector as a positive control. As expected, despite its efficient expression (Fig. 1f), D366N supported HIV-Luc infection at a level that was indistinguishable from the negative control (Fig. 1d, e) [8]. The multiplicity of infection in this experiment was moreover varied to determine if a lower level of input virus might uncover defects in polymorphic mutant behavior. Each of the mutant proteins supported HIV-Luc infection at a level that was equal to or exceeded that of WT LEDGF/p75 (Fig. 1d–f).

We conclude that human polymorphic variants I436S, Q472L, and T473I support efficient HIV-1 infection and integration under these conditions. Because two of the identified mutations abut one another in the primary sequence, and Q472L was previously shown to trend with lower viral loads [10], it is tempting to speculate that the changes nevertheless influence disease progression in ways that are not evident by bulk infectivity measure. Because initial RNAi knockdowns that failed to affect virus titer nonetheless altered the specificity of HIV-1 integration [16], the mutations may subtly alter integration specificity such that overall virus titer is unaffected *ex vivo*. The mutations moreover coincide with two predicted helix-turn-helix motifs that marginally reduce transcriptional co-activator activity *in vitro* [17] (Fig. 1a), suggesting that subtle effects on viral and/or host gene expression might potentially affect disease progression *in vivo*.

Acknowledgments

J.E. and A.E. designed the study, Y.K. and E.B. performed the experiments, Y.K., E.B., J.E., and A.E. analyzed the data, and Y.K., E.B., J.E., and A.E. wrote the paper.

Financial support: This work was supported by US National Institutes of Health grant AI039394 (to A.E.) and the Spanish Ministerio de Economia grants BFU2009-06958 and SAF2012-31569 (J.E.).

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Fig. 1. Ex vivo activities of polymorphic LEDGF/p75 variants

(a) Human LEDGF/p75 schematic showing locations of PWWP domain, nuclear localization signal (NLS), AT-hook DNA binding motifs, charged regions (CR) 1–3, IBD, and helix-turn-helix (H-T-H) motifs [5, 6, 17]. Note the overlap between the IBD and N-terminal H-T-H. The studied mutations are noted. (b) The indicated transfected cells were infected with 10⁶ reverse transcriptase (RT) counts per minute (cpm) of HIV-Luc, which corresponds to an approximate multiplicity of infection of 0.06 [18]. (c) Re-graph of panel (b) after subtracting LEDGF/p75-independent activity. (d and e), same as in panels (b) and (c), except that E6(–/–) cells were infected with 10⁵ RT-cpm (black bars) and 10⁶ RT-cpm. (f) Anti-HA immunoblot of panel (d) transfected cells; β-actin was blotted as loading control. Results in panels (b)–(e) are mean values and standard deviations obtained from duplicate infection, luciferase, and Bradford assays.