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Involvement of LIM kinase 1 in actin polarization in human CD4 T cells

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Chemokine binding to cognate receptors induces actin dynamics that are a major driving force for T cell migration and chemotactic motility. HIV-1 binding to the chemokine coreceptor CXCR4 initiates chemotactic signaling, mimicking chemokine-induced actin dynamics to facilitate infection processes such as entry, early DNA synthesis, and nuclear migration. Recently, we identified that HIV-triggered early actin polymerization is mediated through the Rac1-PAK1/2-LIMK1-cofilin pathway. Inhibition of LIMK1 (LIM domain kinase 1), a kinase phosphorylating cofilin, through shRNA knockdown decreases actin polymerization and T cell chemotaxis toward SDF-1. The LIMK1 knockdown T cells also supported lower viral entry, DNA synthesis and nuclear migration, suggesting a critical role of LIMK1-mediated actin dynamics in the initiation of HIV-1 infection. Surprisingly, LIMK1 knockdown in CEM-SS T cells did not lead to an overall change in the ratio of phospho-cofilin to total cofilin although there was a measurable decrease in the amount of actin filaments in cells. The decrease in filamentous actin in LIMK1 knockdown cells was found to mainly occur in polarized cap region rich in F-actin. These results suggest that LIMK1 may be involved in spontaneous actin polarization in transformed T cells. The inhibition of T cell chemotaxis by LIMK1 knockdown likely result from inhibition of localized LIMK1 activation and cofilin phosphorylation that are required for polarized actin polymerization for directional cell migration. The inhibition of HIV-1 infection by LIMK1 knockdown may also result from the decrease of actin-rich membrane protrusions that may be preferred viral entry sites in T cells.

Keywords: LIMK1, cofilin, chemotaxis, SDF-1, CXCR4, HIV-1, CD4 T cells, Rac1, Pak1, Pak2

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HIV-1 entry into CD4 T cells is mediated through viral envelope binding to CD4 and the chemokine coreceptor CXCR4.[1,2] This interaction is required for viral fusion with the plasma membrane. HIV-1 binding to these receptors also initiates signal transduction in T cells.[3] In particular, HIV-1-mediated signal transduction from the chemokine coreceptor CXCR4 has been shown to trigger actin dynamics critical for viral entry, post entry DNA synthesis and nuclear migration.[4-6] It is suggested that in the absence of chemotactic stimulation or T cell activation, the cortical actin in blood resting CD4 T cells is relatively static. This lack of actin activity hinders viral intracellular migration across the actin cortex following fusion. To overcome this limitation, HIV-1 uses CXCR4 signaling to trigger the activation of cofilin, promoting actin treadmilling and viral nuclear migration.[4] The importance of actin dynamics in HIV-1 infection has also been highlighted by several recent studies. Induction of the actin activity by treatment of blood CD4 T cells with chemokines such as CCL2 augments gp120-induced F-actin polymerization, which enhances viral DNA synthesis.[7] Pretreatment of blood CD4 T cells with CCL19, CXCL9, CXCL10 and CCL20 triggers cofilin activation and changes in actin filaments, which promote viral nuclear localization and DNA integration.[8-10] In addition, spinoculation, or infecting CD4 T cells under the condition

of centrifugal stress, triggers both cofilin activation and actin dynamics, leading to CXCR4 upregulation and a great enhancement of HIV-1 DNA synthesis and nuclear migration.[11]

Mechanistically, HIV-1-mediated actin dynamics have been implicated in several early processes in the initiation of HIV infection. Actin binding proteins such as filamin-A and moesin were identified as possible cofactors involved in HIV-1 entry. Filamin-A may anchor CD4 and CXCR4 to F-actin following receptor clustering.[12] The Ezrin-Radaxin-Moesin (ERM) family protein moesin is also suggested to promote CD4/CXCR4 receptor clustering following its activation by gp120.[13,14] In addition, HIV gp120-mediated cell-cell fusion has been suggested to extensively rely on signal transduction leading to actin dynamics. siRNAs or inhibitors against molecules such as Pyk2, Rac1, GTPase Ras, phospholipase C, protein kinase C, Tiam-1, Abl, IRSp53, Wave2 inhibited gp120-medaited cell-cell fusion.[15] Following viral entry, the establishment of an active viral reverse transcription complex may also involve cytoskeletal actin.[16] Multiple proteins in the viral preintegration complex are identified to interact with actin. These proteins include the gag nucleocapsid protein (NC),[17-20] the large subunit of the viral reverse transcriptase, the viral integrase and Nef.[21-24] In addition, HIV-1 intracellular migration and nuclear localization is suggested to be dependent on actin treadmilling mediated by cofilin activity.[4]

Recently, we also found that HIV-1 binding to resting CD4 T cells triggers a rapid and transient actin polymerization through Rac1-PAK1/2-LIMK1 activation.[6] Functionally, HIV-mediated actin polymerization may be required to transiently block CXCR4 internalization for the stabilization of the fusion complex. In addition, LIMK1-mediated actin polymerization is involved in HIV-1 reverse transcription and nuclear migration, as knockdown of LIMK1 inhibited both viral DNA synthesis and nuclear migration. Furthermore, transient treatment of resting CD4 T cells with a pharmacological agent, okadaic acid, activates LIMK1 and promotes HIV-1 latent infection of resting CD4 T cells.[6]

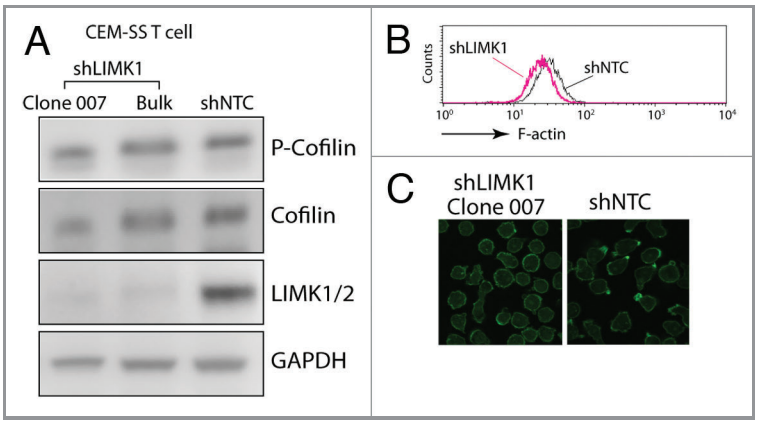

Figure 1. Involvement of LIMK1 in actin polarization in human CD4 T cells. (A) shRNA-mediated LIMK1 knockdown in CEM-SS T cells. Cells carrying stable LIMK1 knockdown or shNTC (a control shRNA against no human genes) were selected in puromycin and analyzed by western blot using antibodies against human phospho-cofilin, cofilin, LIMK1/2 or GAPDH (Bulk, bulk cell populations; clone 007, a derived LIMK1 knockdown cell clone). (B and C) LIMK1 knockdown decreases F-actin in clone 007. The decreases of F-actin in the knockdown cells were measured by FITC-phalloidin staining and flow cytometry (B) or by confocal microscopy imaging (C).

Given that LIMK is the kinase responsible for the serine 3 phosphorylation of cofilin, which inhibits cofilin activity and its binding to actin filaments,[25,26] we expected that knockdown of LIMK1 would lead to an increase in cofilin activity and a decrease in the cortical actin density, as opposed to what we have seen in cofilin knockdown T cells.[4] Indeed, LIMK1 knockdown led to a slight decrease of the F-actin intensity in CEM-SS T cells, as judged by flow cytometry[6] (**Fig. 1B**). However, when the status of phospho-cofilin was examined, we did not see a measurable change in the ratio of phospho-cofilin to total cofilin (**Fig. 1A**). Further examination of the cortical actin staining by confocal microscopy revealed that there was a marked decrease of the F-actin-rich protrusions in the LIMK1 knockdown cells, whereas these spontaneous polarized actin-rich caps were often observed in the control knockdown cells (shNTC) (**Fig. 1C**). This observation prompted us to hypothesize that in cycling CEM-SS T cells where cofilin activity is constantly required for cytoskeletal remodeling, the maintenance of basal levels of cofilin phosphorylation in the absence of chemotactic stimulation may involve only small amounts of LIMK1. LIMK1 may also be locally enriched in regions where polarized actin caps are localized. Thus, decreasing LIMK1 activity by shRNA knockdown may locally increase cofilin activity, destabilizing these actin-rich caps. Upon chemotactic stimulation, rapid actin polymerization mainly occurs around polarized lamellipodium at the leading edges of migrating cells; rapid LIMK1 activation would be required for transient phosphorylation of cofiin in these locations.[27] Thus, a decrease of LIMK1 activity may impair the polarization of actin-rich caps and the ability of T cell for directional migration.[6,28] For HIV-1 infection, it has been suggested that actin-rich membrane protrusions such as microvilli may be prefer sites for viral binding and entry in T cells.[29] It is attempting to speculate that knockdown of LIMK1 may also result in the decrease of these actin-rich protrusions, leading a less efficient entry and less viral contact with the cytoskeletal actin. This speculation certainly deserves further detailed studies in the future.

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