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Focal Adhesion Proteins Talin-1 and Vinculin Negatively Affect Paxillin Phosphorylation and Limit Retroviral Infection

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Many of the early events in retroviral infection are not well understood, but it is known that the host cytoskeleton and signaling pathways play integral roles in various entry and post-entry processes. Focal adhesion complexes act as sites of integration for both cytoskeletal organization and integrin signaling at the cell surface. Here, we show that talin-1 and vinculin, two interacting proteins that localize in focal adhesions to mediate integrin linkage to the actin cytoskeleton, function during retroviral infection. Transient overexpression of either talin-1 or vinculin reduced the susceptibility of human cells to infection with pseudotyped human immunodeficiency virus type 1 (HIV-1) and Moloney murine leukemia virus. In contrast, transient short interfering RNA-mediated knockdown of talin-1 or vinculin increased infection by pseudotyped HIV-1 and simian immunodeficiency virus, demonstrating that the endogenous forms of these proteins also impaired retroviral infection. Talin-1 or vinculin overexpression inhibited infection by retroviruses that entered the cell by either fusion or endocytosis, while analysis of HIV-1 DNA synthesis demonstrated that the block occurred early in infection and prior to the initiation of reverse transcription. Both factors retained antiviral activity in the presence of actin or microtubule depolymerizing agents. Finally, talin-1 and vinculin expression was found to negatively influence tyrosine phosphorylation of paxillin, a major focal adhesion scaffolding protein whose transient knockdown decreased pseudotyped HIV-1 infection. Together, these findings demonstrate that talin-1 and vinculin negatively affect tyrosine phosphorylation of paxillin, a novel positive regulator of

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Abbreviations used: HIV-1, human immunodeficiency virus type 1; M-MuLV, Moloney murine leukemia virus; SIV, simian immunodeficiency virus; VSV-G, vesicular stomatitis virus; MT, microtubule; FAK, focal adhesion kinase; ECM, extracellular matrix; ERM, ezrin/radixin/moesin; ERK, extracellular signal-regulated kinase; siRNA, short interfering RNA; GFP, green fluorescent protein; MSS, minus-strand strong stop; DMSO, dimethyl sulfoxide; FERM, 4.1/ezrin/radixin/moesin; KS, Kaposi's sarcoma; qPCR, quantitative real-time PCR; TBS-T, Tris-buffered saline and 1% Tween.

HIV-1 infection, and impose an early block to infection by distinct retroviruses.

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Introduction

As intracellular parasites, viruses must manipulate many cellular functions to complete their life cycle, including the activity of their hosts' cytoskeletal networks and kinase signaling pathways. The host cytoskeleton plays a complex role in many stages of infection. Many retroviruses surf along the cell surface using an actin-mediated process to find a suitable site of entry. To enter the cell, they then need to penetrate through the layer of cortical actin that lies beneath the cell surface and that functions both as a facilitator and as a barrier to the entry of incoming viruses.[1–3] Upon entry into the cell, it is known that viruses use the host cytoskeleton to reach specific cellular locations at the correct stage in the viral life cycle to facilitate their replication, yet the exact trafficking mechanisms used are not well understood.[4,5] Soon after entry into the cytoplasm, the viral core is uncoated and the viral RNA genome is reverse transcribed, although the exact order and mechanism(s) involved in these processes remain somewhat controversial. It is thought that reverse transcription of the human immunodeficiency virus type 1 (HIV-1) genome is completed before nuclear entry and has recently been suggested to occur at the nuclear pore.[6,7] It has also been shown that the HIV-1 reverse transcription complex associates with the cytoskeleton and that efficient reverse transcription is dependent on functional actin dynamics.[8] The pre-integration complex may also associate with actin networks, as direct interactions of viral nucleocapsid, reverse transcriptase and integrase with actin have all been described.[9–11] Subsequent movement of incoming viral cores toward the nucleus exploits the host cells microtubule (MT) network, a cytoskeletal system for the long-range transport of macromolecules.[12,13] The processes that govern the movement of incoming cores and the factors involved remain poorly understood. Recently, the ezrin/radixin/moesin (ERM) family members moesin and ezrin that mediate linkage of the plasma membrane to actin filaments were found to also negatively regulate the formation of a small subset of stable detyrosinated MTs (termed Glu-MTs) and impose an early post-entry block to infection by both murine leukemia virus and HIV-1 in various mammalian cells.[14,15] In-line with these findings, virus-envelope-mediated increases in the levels of stabilized acetylated MTs have also been suggested to play a role in HIV-1 cell fusion and infection.[16] In addition, IQGAP1, another regulator of actin and MT networks, has been shown to directly interact with murine leukemia virus matrix protein and affects both the early and late stages of viral infection.[17] Overexpression of the MT-motor-associated factor FEZ1 blocks the transport of retroviral DNA into the nucleus after reverse transcription,[18] while the kinesin motor protein KIF4 associates with retroviral Gag and functions in the outward movement of viral particles.[19–21] As such, proteins that associate with or regulate actin and/or MT function are likely to play important roles at a variety of stages in the retroviral life cycle.

In addition to the host transport machinery, retroviruses also hijack critical signaling pathways to facilitate their replication. HIV-1-envelope-mediated signaling events that regulate actin cytoskeleton dynamics have recently been suggested to promote early post-entry steps of viral infection in immune cells.[22,23] HIV-1-envelope-mediated activation of signal transduction pathways is also known to regulate intracellular calcium mobilization and PI3K-dependent activation of all three mitogen-activated protein kinase family members (ERK1/2, JNK, and p38) and focal adhesion kinase (FAK).[24] FAK phosphorylates paxillin, a key component of integrin signaling in focal adhesions, which are large dynamic protein complexes that attach the cytoskeleton to the extracellular matrix (ECM) and play an important role in cell signaling and motility.[25] Tyrosine phosphorylation of paxillin generates docking sites for diverse cytoskeletal and signaling factors.[26] As focal adhesion proteins are important for the transfer of force from the ECM through the host cytoskeleton and in regulating signaling pathways from the cell surface, they are good candidates for facilitating or obstructing early events in viral infection. Indeed, FAK activity and focal adhesion assembly have previously been suggested to be involved in HIV-1 infection.[27–33]

In this study, we investigated the effects of talin-1 and vinculin, two proteins that localize to focal adhesions, on retroviral infection both by transient overexpression of these proteins and by short interfering RNA (siRNA)-mediated reduction in the expression of their endogenous forms. We found that under both conditions, talin-1 and vinculin had negative effects on infection by a variety of pseudotyped retroviruses in a number of different human cell lines, and these negative effects occurred independently of the route of viral entry. It was also found that the block to infection occurred early in the viral life cycle, before the initiation of reverse transcription. While disruption of actin or MT polymerization did not affect the antiviral activity of talin-1 or vinculin, expression of these proteins was