PDZD8 Is a Novel Gag-Interacting Factor That Promotes Retroviral Infection

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In a yeast two-hybrid screen for cellular factors that could interact with human immunodeficiency virus type 1 (HIV-1) Gag protein, we identified PDZD8 and confirmed the interaction by coimmunoprecipitation (co-IP). PDZD8 overexpression promoted the initiation of reverse transcription and increased infection by pseudotyped retroviruses independent of the route of viral entry, while transient knockdown of endogenous levels decreased HIV-1 infection. A mutant of PDZD8 lacking a predicted coiled-coil domain in its Gag-interacting region failed to bind Gag and promote HIV-1 infection, identifying the domain of PDZD8 required for mediating these effects. As such, we identify PDZD8 as a novel positive mediator of retroviral infection.

Like all viruses, retroviruses are dependent upon multiple host cell functions during their life cycle (14). As a result, mammalian cells have evolved a species-specific antiviral response mediated by proteins called restriction factors, which form what is now referred to as the intrinsic immune system (2, 40). In addition to these restriction factors, whose only well-characterized function to date is to block viral infection, other cellular factors and pathways that affect various stages of the retroviral life cycle have also been reported (14, 17, 30). Indeed, recent RNA interference (RNAi)-based library screens have identified various host factors that influence cellular sensitivity to retroviral infection (5, 24, 42, 44), highlighting the complex interplay between the host and retroviruses and the potential for developing new antiviral strategies.

An attractive therapeutic target is the retroviral structural polyprotein Gag, which functions in both early and late stages of retroviral infection (27). We therefore performed a yeast two-hybrid screen (13) of a human testis cDNA prey library using HIV-1 N538/BRU Gag as bait. The residues of Gag (amino acids [aa] 39 to 250) used in the screen contained the C terminus of matrix (MA) and the N terminus of capsid (CA), a fragment of Gag that expresses well in yeast and folds correctly, and were used to identify host factors capable of interacting with either of these important structural components of the virus. From this screen we isolated PDZD8 (aa 932 to 1119) as a strongly interacting protein (data not shown). PDZD8 is a 1,154-aa protein that contains a PDZ domain (aa 366 to 449), a zinc finger domain (aa 840 to 891), and a region predicted to form a coiled-coil motif (aa 1028 to 1063). PDZD8 is poorly understood but belongs to a larger group of proteins containing conserved PSD-95/DlgA/ZO-1-like (PDZ) interaction domains (22), which bind membrane and other PDZ domain-containing proteins and function in cytoskeletal organization and protein trafficking (12, 18, 33).

To study PDZD8 further, we generated human brain microglia CHME3 cells (19), a natural target cell type for human immunodeficiency virus type 1 (HIV-1) involved in the development of HIV-associated dementia (15), stably overexpressing tagged human PDZD8. To do this, transducing murine leukemia viruses (MuLV) were generated by cotransfection of 293T cells with the pQCXIN (Clontech) vector containing full-length C-terminally Flag-tagged human PDZD8 (OriGene Technologies) or Flag control, along with constructs expressing MuLV Gag-Pol and vesicular stomatitis virus G (VSV-G) envelope protein (29). CHME3 cells were infected, and stable neomycin-resistant PDZD8-expressing or Flag control cell lines were isolated. The PDZD8-Gag interaction was then tested by transfecting these cells with HIV-1 Gag-Pol-expressing vector p8.91 (31), and soluble cell extracts were RNase treated, precleared, and immunoprecipitated using anti-Flag antibody (38). Immunoglobulin complexes were recovered on protein A-Sepharose, washed extensively, and boiled in sample buffer. Input and immunoprecipitated samples were analyzed by Western blotting (WB) using anti-HIV-1 Gag (7, 37, 39) or anti-Flag antibodies. While the Gag polyprotein p55 and PDZD8 were detected in immune complexes recovered from cells expressing Flag-tagged PDZD8, neither protein was found in the control samples from cells expressing Flag alone (Fig. 1A), despite efficient expression of p55 in both control and PDZD8 cells (Fig. 1B). Equal levels of expression and loading were confirmed by examining the endogenous cellular protein eIF4E (Fig. 1B). This demonstrated that PDZD8 and Gag interacted in mammalian cells, confirming the initial yeast two-hybrid identification.

To determine whether this newly identified HIV-1 Gag-interacting factor had any functional significance in HIV-1 replication, we first examined whether overexpression of PDZD8 affected the sensitivity of human cells to HIV-1 infection. CHME3 cells stably overexpressing Flag-tagged PDZD8 or Flag alone were infected with HIV-1-VSV-luc and HIV-1-VSV-puro, HIV-based viral vectors pseudotyped with the VSV-G envelope protein carrying a luciferase reporter or a puromycin resistance marker, respectively (16). Overexpres-
sion of PDZD8, as illustrated by two of the tested Flag-tagged PDZD8 clones, enhanced infection by either HIV-1-VSV-luc (Fig. 1C) or HIV-1-VSV-puro (Fig. 1D) relative to the control line expressing Flag alone. When the levels of exogenously expressed PDZD8 were determined by WB using anti-Flag antibody (Fig. 1E), its expression correlated with the degree of infection in each line, demonstrating that PDZD8 promoted HIV-1 infection.

The sequences within PDZD8 interacting with HIV-1 MA/CA isolated from the yeast two-hybrid screen contained a predicted coiled-coil motif. Several host factors have been reported to interact with retroviral MA and CA, some of which occur via coiled-coil domains (8, 10, 11, 14, 20, 27). To examine the potential functionality of this predicted coiled-coil region within PDZD8, C-terminally truncated (aa 1 to 1028) Flag-tagged PDZD8 was generated by PCR using pQCXIN, described above as a template. The PCR product was ligated into the mammalian expression vector pCDNA3.1+/H11002, and cloning fidelity was confirmed by sequencing. 293A cells were then transfected with pCDNA3.1+/H11002 vectors containing C-terminally Flag-tagged full-length or truncated (lacking residues 1028 to 1155, including the predicted coiled-coil residues) forms of PDZD8 using FuGENE-HD transfection reagent (Roche). Forty-eight hours posttransfection, cultures were infected with HIV-1-VSV-luc. While transient overexpression of full-length PDZD8 promoted HIV-1 infection, 293A cells overexpressing...
the truncated PDZD8 variant or empty vector control were equally susceptible to infection (Fig. 1F). WB using anti-Flag antibodies confirmed equal expression levels of the PDZD8 variants, while eIF4E served as a loading control (Fig. 1G). This demonstrated that the putative C-terminal coiled-coil motif in the Gag-interacting region of PDZD8 was required to promote HIV-1 infection.

To determine whether this region of PDZD8 was also responsible for mediating its interaction with HIV-1 MA/CA, coimmunoprecipitation (co-IP) analysis was performed as described above. 293A cells were transiently cotransfected with p8.91 along with equimolar amounts of the control pcDNA3.1/H11002, Flag-tagged full-length PDZD8, or truncated PDZD8 constructs, and cell extracts were immunoprecipitated using anti-Flag antibody as described above. Input and immunoprecipitated samples were analyzed by WB using anti-HIV-1 Gag (7, 37, 39) or anti-Flag antibodies. While Gag polyprotein p55 and PDZD8 were readily detected in immune complexes recovered from cells expressing Flag-tagged full-length PDZD8, or truncated PDZD8 constructs, and cell extracts were immunoprecipitated using anti-Flag antibody as described above. Input and immunoprecipitated samples were analyzed by WB using anti-HIV-1 Gag (7, 37, 39) or anti-Flag antibodies. While Gag polyprotein p55 and PDZD8 were readily detected in immune complexes recovered from cells expressing Flag-tagged full-length PDZD8, neither protein was found in samples from control cells (Fig. 1H), despite efficient expression of p55 (Fig. 1I). Notably, p55 was no longer found in immune complexes from cells expressing Flag-tagged truncated PDZD8, despite efficient expression and immunoprecipitation of truncated PDZD8 protein (Fig. 1H) and equal levels of expression of p55 and PDZD8 in all input samples (Fig. 1I). Equal levels of sample extraction and loading were further confirmed by WB analysis of input samples with antibody toward the endogenous cellular protein eIF4E (Fig. 1I). These results demonstrated that the same region of PDZD8 that mediated increased susceptibility to infection also mediated its interaction with HIV-1 Gag.

To determine the step in the viral life cycle at which PDZD8 functioned, viral DNA synthesis was examined after infection...
of PDZ-Flag-1.3 and Flag-1.2 lines with HIV-1-VSV-puro (16). Low-molecular-weight DNA was isolated 24 h postinfection (h.p.i.) and used as a template for quantitative reverse transcription (RT)-PCR (qPCR) using primers to amplify the first detectable viral DNA, minus-strand strong-stop (MSS) DNA (4), or total viral DNA (16). PDZD8-overexpressing CHME3 cells were found to contain increased levels of both forms of viral DNA (Fig. 2A and B) compared to the control line, suggesting that overexpression of PDZD8 affects HIV-1 infection at an early stage.

As different PDZ domain-containing proteins have been shown to play a role in infection by several retroviruses (3, 21, 25, 32), we also tested the effects of PDZD8 on MuLV and simian immunodeficiency virus (SIV) infection. Control and PDZD8-overexpressing CHME3 cells were found to contain increased levels of both forms of viral DNA (Fig. 2A and B) compared to the control line, suggesting that overexpression of PDZD8 affects HIV-1 infection at an early stage.

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As the retroviruses tested were all pseudotyped with VSV-G envelope protein, entering the cell by endocytosis, we also tested whether the effect of PDZD8 was dependent on the route of viral entry. To do this, PDZ-Flag-1.3 and Flag-1.2 lines were infected with HIV-1-ampho-luc, an HIV-based viral vector carrying a luciferase reporter pseudotyped with an amphotropic envelope protein, which enters the cell by plasma membrane fusion. Again, PDZD8 overexpression increased the susceptibility of CHME3 cells to infection (Fig. 3C), suggesting that PDZD8 functions independently of the route of retroviral entry.

To test whether endogenous levels of PDZD8 also influence cellular susceptibility to infection, different human cell lines were treated with two independent short interfering RNA (siRNA) duplexes toward human PDZD8 (Ambion) or a control duplex, and the consequences were assessed by infection with HIV-1-VSV-luc (16). In both CHME3 and 293A cells, PDZD8-specific RNAi duplexes significantly reduced expression of PDZD8, as confirmed by qPCR (Fig. 4A and B, respectively) and resulted in significantly reduced infection by
HIV-1-VSV-luc compared to the control duplex (Fig. 4C and D, respectively). As such, these findings demonstrated that endogenous levels of PDZD8 also functioned to enhance HIV-1 infection of different human cell types.

PDZ domain-containing proteins are a family of multidomain scaffold proteins that function in the assembly of supramolecular complexes involved in protein trafficking and cytoskeletal organization (12, 18, 33). Members have recently been shown to play a role in papillomavirus and adenovirus infection (21, 36). Another PDZ protein, Dlg1 (discs large protein), directly binds HIV-1 Gag and negatively regulates late steps of the viral life cycle (32). Dlg1 also interacts with human T-cell leukemia virus type-1 envelope glycoprotein and regulates cell-cell fusion in T lymphocytes (3). Furthermore, interaction between HIV-1 trans-activator protein Tat and the PDZ-like domain of SATB1 (special AT-rich sequence binding protein 1) has been suggested to function in viral propagation in T cells (25). These findings suggest that PDZ domain-containing proteins play important roles at various stages of distinct viral infections.

Notably, the biological function of PDZD8 is not known. The only report to date identifies PDZD8 as a potential candidate gene for the forearm-girdle muscular anomaly disorder reported for Japanese black cattle (28). As such, our findings provide the first direct demonstration of a function for PDZD8, facilitating early postentry events in retroviral infection. The C terminus of PDZD8 directly associates with the HIV-1 Gag-containing regions of MA and CA. Notably, the same domain is required for its effects on retroviral infection, as detected in our single-round infection assays, suggesting that the interaction of PDZD8 with MA and/or CA of the incoming core is critical to its function in promoting infection. Both MA and CA are involved in early postentry events of the retroviral life cycle (9, 14, 23, 34). The N-terminal domain of CA has been reported to be of importance for core stability and the initiation of HIV-1 reverse transcription (35), while the interaction of MA with actin microfilaments has been suggested to activate the reverse transcription complex (6). The interaction of MA with IQGAPs, cytoskeleton regulators involved in trafficking of virus both in and out of the cell, also affects the initiation of M-MuLV reverse transcription (26). As with PDZD8, interactions with unprocessed HIV-1 Gag polyprotein and the incoming CA have been reported for cyclophilin A, another cellular factor required for early events in the infection of human cells (34). As such, numerous host factors interact with components of incoming cores to influence infection. PDZD8, through its coiled-coil-containing C terminus, may facilitate the retroviral core’s interaction with other proteins or directly influence core function or trafficking upon entry into the cell. Although this remains to be determined, our findings, together with others’ (10, 20, 34), suggest that a number of coiled-coil domain-containing proteins play critical roles in mediating host protein interactions with viral cores that affect early postentry processes during retroviral infection. In the case of proteins such as PDZD8 that do not appear to have critical functions within the host cell, targeting the dependence of the virus on these host factors could provide a means to inhibiting viral infection that circumvents the emergence of the drug resistance associated with targeting viral proteins.

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