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Pth15: An Interfacial Inhibitor of HIV-1 Integrase That Efficiently Blocks HIV-1 Replication

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HIV-1 integrase (IN) is currently one of the major pharmacological targets for the development of new anti-HIV drugs. Most of the IN inhibitors described so far essentially target its enzymatic activities. In order to elaborate novel specific inhibitors of IN, we have developed a strategy based on short peptides that target major protein–protein interfaces required for IN activation and its implication in the pre-integration complex. We have designed a 15-mer peptide, Pth15, originally derived from the Thumb subdomain of HIV-1 reverse transcriptase that forms stable complexes with a Kd in the nanomolar range, with both recombinant and cellular HIV-1 IN. We have demonstrated that this peptide inhibits both 3′ processing and strand-transfer activities of IN in vitro. Moreover, in contrast to most IN inhibitors, low micromolar concentrations of Pth15 not only prevent association of IN with its DNA substrate, but also promote dissociation of preformed IN-DNA complexes. In order to evaluate the potency of Pth15 to block viral replication in cellulo and to circumvent problems related to the poor cellular uptake, Pth15 was complexed with the cell-penetrating peptide Pep1. Pep1-Pth15 nanoparticles enter cells efficiently and inhibit HIV-1 replication robustly in MT4 cells infected with HIV-LAI, with EC_{50} values in the nanomolar range and a selectivity index of about 3000. From a mechanistic point of view, we have demonstrated that Pth15 significantly affects the nuclear localization of IN and that its major mechanism involves dissociating interactions between IN and its viral or cellular partners. Whether Pth15 disrupts the interaction between IN and proteins responsible for its nuclear retention or instead prevents IN association with partners involved in its nuclear import remains to be addressed. Hence, our study demonstrated, for the first time, that a short peptide targeting protein–protein interfaces within the pre-integration complex can efficiently inhibit HIV-1 replication. This work validates the consistency of an interfacial inhibitor strategy, and opens new perspectives for the targeting of therapeutic drugs.

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Effective Small Interfering RNAs Targeting AlphaV Integrin Inhibit HIV-1 Replication

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Integrins are heterodimeric cell surface receptors, that play a key role as cell adhesion molecules. Many viruses are capable of exploiting host cell surface integrins during their replication cycles, with a variety of different mechanisms. Previous data suggest an involvement of alphaV integrin in the replication of HIV-1 in monocyte-derived macrophages (MDM). However, little is known on the step and alphaV integrin-mediated mechanism influencing HIV-1 replication. RNA interference (RNAi) is a powerful tool to silence gene expression, and therefore useful to analyze the functions of genes. We have developed different small interfering RNAs (siRNAs) specifically targeting alphaV integrin gene in order to study the role of alphaV integrin in HIV-1 replication in HeLa-MAGI cells. Using the siRNAs, a high level of alphaV silencing was achieved in HeLa-MAGI cells. AlphaV silencing was linked to a morphological changed without affecting cellular viability. Similar results were obtained using a synthetic integrin antagonist (S36578-2) selective for avb3 and avb5 integrins. Replication of HIV-1 was inhibited in alphaV-silenced HeLa-MAGI cells, as well as in the presence of S36578-2, thus confirming a role for alphaV integrin in the HIV-1 replication cycle. Supporting these data, S36578-2 antiviral activity was lost when HeLa-MAGI cells were cultured in low-adhesive conditions. Moreover, S36578-2 inhibited Tat transactivation of HIV-1 promoter, suggesting that integrin-mediated signalling would influence HIV replication at a transcriptional level. In summary, by using RNAi we have confirmed a role of alphaV integrin in the replication cycle of HIV-1.

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