Zinc-finger endonuclease targeting LEDGF/p75 inhibits HIV-1 integration

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ABSTRACT

BACKGROUND: Human immunodeficiency virus (HIV) requires several host factors to successfully complete viral replication cycle. Genome editing using zinc-finger nucleases (ZFN) has been successfully applied to disrupt CCR5 or CXCR4 host factors, inhibiting viral entry and infection. Lens epithelium-derived growth factor (LEDGF/p75) is a lentivirus-specific cellular cofactor which directly interacts with viral integrase, tethering viral preintegration complex into active transcription units of the cellular chromatin. Gene therapy using ZFN to modify LEDGF gene might restrain a late step of viral replication cycle at the integration level.

METHODOLOGY: Knockout ZFNs targeting the *PSIP1* gene (ZFN_{LEDGF}) were designed to specifically target the sequence nearby the Integrase Binding Domain (IBD) of LEDGF/p75 protein. TZM-bl cells were transfected with ZFN_{LEDGF} expressing plasmids carrying a ZsGreen reporter gene. Moreover, LEDGF/p75 knock-out cells were infected with HIV-1 R5 tropic BaL and the X4 tropic NL4.3 strains and infection was monitored by proviral and integrated viral DNA at 24h post infection and β-galactosidase assay 3 days after infection. AZT, AMD3100 and raltegravir were used as controls.

RESULTS: A total of 15% of TZM-bl ZFN_{LEDGF} positive cells were obtained after transfection and cell sorting of GFP expressing cells. Assessment of ZFN_{LEDGF} efficiency in the deletion of the gene by heteroduplex formation and DNA sequence analysis, revealed two homozygotic cell clones with deletions ranging from 17 to 41 bp surrounding the target sequence of ZFN_{LEDGF}. We confirmed that ZFN_{LEDGF} successfully recognized the target region of the LEDGF gene, induced a frame shift of the coding region and resulted in the abolishment of LEDGF expression at mRNA and protein level. Functional assays revealed that infection with R5 BaL or X4 NL4.3 viral strains was impaired in LEDGF knock-out cells regardless of entry tropism (up to 85% inhibition, p<0.05). Disruption of gene coding for LEDGF/p75 protein restricted HIV-1 provirus integration into host genome. However, residual infection was detected in LEDGF knock-out cells. Indeed, LEDGF knock-out restriction was overcome at high virus multiplicity of infection, suggesting alternative mechanisms for HIV-1 genome integration rather than LEDGF/p75. Observed residual integration was, however, sensitive to the integrase inhibitor raltegravir.

CONCLUSION: Genome editing is an incoming issue for HIV-1 research and treatment. Here, we describe a novel ZFN that effectively targets *PSIP1* gene which is involved at late steps of viral replication cycle. LEDGF knock-out cells represent potent tools to elucidate the role of HIV integration cofactors in virus replication. The ZFN_{LEDGF} becomes a potential antiviral agent to restrict HIV-1 integration.

1.- DESIGN OF THE ZFN_{LEDGF} , EXPERIMENTAL STRATEGY AND ISOLATION OF LEDGF/p75 KNOCK-OUT CELLS

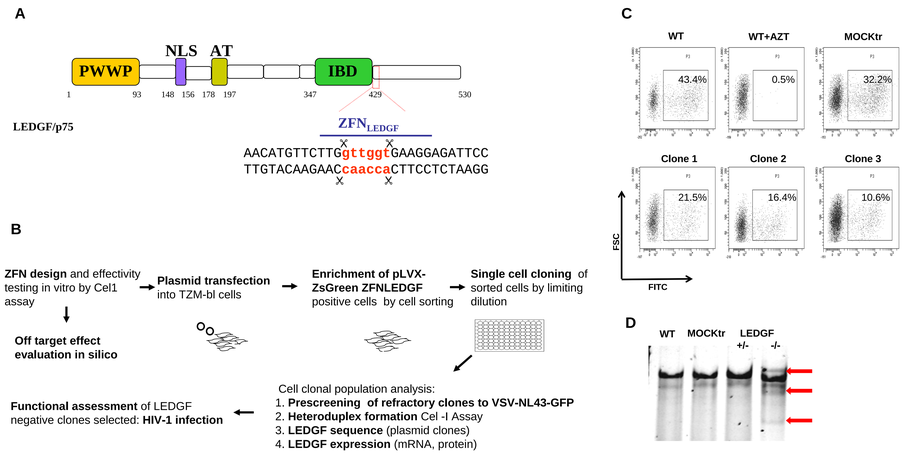


Figure 1. Experimental design and specificity of the ZFNLEDGF targeting *PSIP1* gene.
A. Schematic representation of *PSIP1* gene highlighting the position of the ZFN_{LEDGF} targeted region. Functional domains of the LEDGF/p75 are mainly located at the N-terminal region of the protein. Cutting site of the ZFN_{LEDGF} is located nearby the sequence coding for the Integrase binding domain of the LEDGF/p75 (IBD) within the C-terminal region of the protein.
B. Experimental design used to generate and evaluate LEDGF/p75 knockout cells.
C. Flow cytometry plots of wild-type, mock-treated cells and cells transfected with ZFN_{LEDGF} plasmids and challenged with VSV-pseudotyped NL4.3-GFP expressing virus. Clones refractrory to VSV-pseudotyped NL4.3-GFP were chosen for the following experiments.
D. Gene editing by ZFN_{LEDGF} induces heteroduplex formation determined by the surveyor mutation assay (Cel-I) in the selected clones.

2.- GENOME EDITION INDUCED BY THE ZFN_{LEDGF}

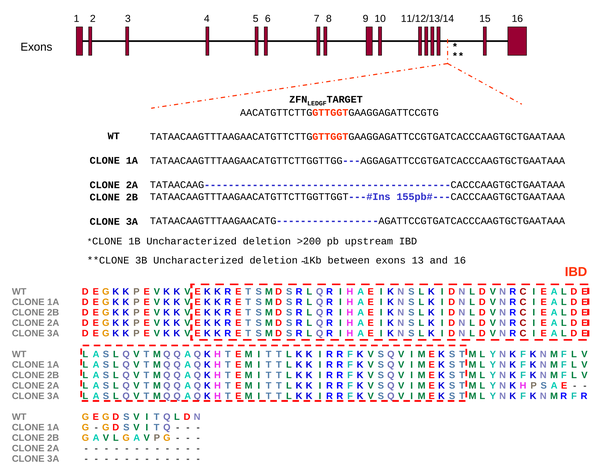


Figure 2. Characterization of genetic modifications induced by the ZFN_{LEDGF} in the selected clones.
A. Sequence analysis of the insertions and deletions product of ZFN_{LEDGF} treatment. At least 16 different *PSIP-1* sequences from the three selected clones were sequenced and aligned. Although Clone 1 has mutations in both alleles, the 3bp deletion in one of them, did not induce a sequence frame shift (see sequence Clone1A). Therefore, clone 1 has a $LEDGF^{+/-}$ phenotype. Clones 2 and 3 with large insertions and/or deletions were identified as homozygotic $LEDGF^{-/-}$ KO mutants.
B. Predicted protein alignment of the selected clones showed that Integrase Binding Domain (IBD) is conserved in the truncated LEDGF/p75 forms induced by the ZFN_{LEDGF}.

3.- GENE EXPRESSION OF *PSIP1* IN THE LEDGF/p75 KNOCK-OUT CELLS

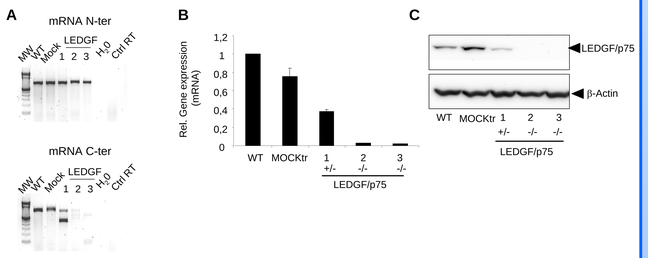


Figure 3. Gene expression of PSIP1 (mRNA/protein in LEDGF/p75 knockout cells.
A. Gene expression of *PSIP1* mRNA corresponding to the N-ter and C-ter regions of the LEDGF/p75.
B. Quantification of the gene expression (mRNA) corresponding to the C-terminal region of the LEDGF/p75 in the selected clones. Expression of LEDGF mRNA was completely inhibited in $LEDGF^{-/-}$ KO clones tested compared to untreated or mock-transfected cell. Heterozygotic $LEDGF^{+/-}$ (clone 1) showed a 50% decrease of the LEDGF mRNA compared to control cells.
C. Assessment of protein levels of LEDGF/p75 determined by Western-blot in the selected LEDGF/p75$^{+/-}$ and LEDGF/p75$^{-/-}$ KO clones compared to control cells.
WT, wild-type, MOCK, mock transfected, Ctrl RT, reverse transcription negative control,

4.- HIV-1 INFECTION IN LEDGF/p75 KNOCK-OUT CELLS

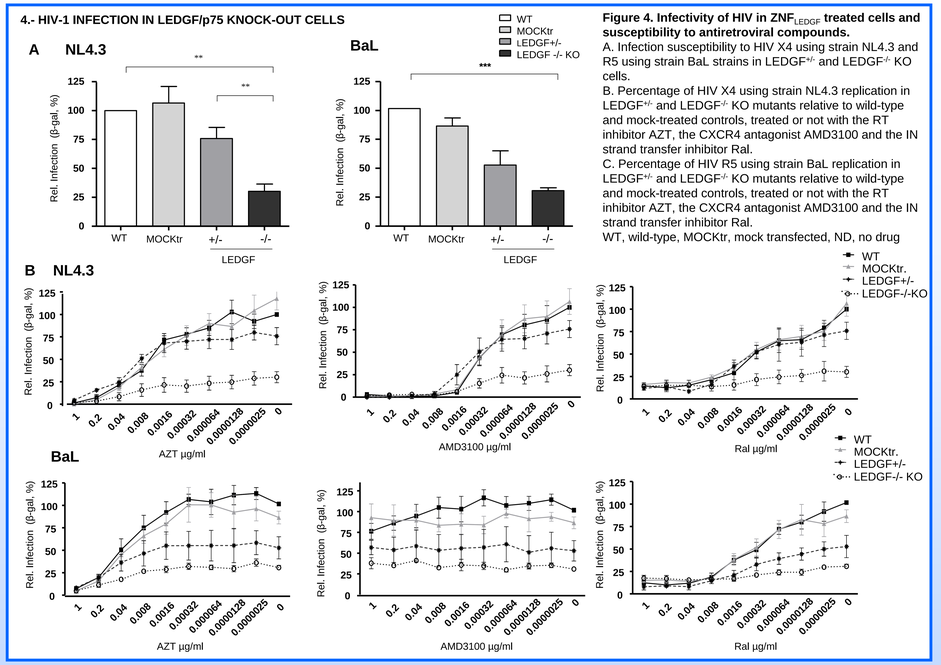


Figure 4. Infectivity of HIV in ZNF_{LEDGF} treated cells and susceptibility to antiretroviral compounds.
A. Infection susceptibility to HIV X4 using strain NL4.3 and R5 using strain BaL strains in $LEDGF^{+/-}$ and $LEDGF^{-/-}$ KO cells.
B. Percentage of HIV X4 using strain NL4.3 replication in $LEDGF^{+/-}$ and $LEDGF^{-/-}$ KO mutants relative to wild-type and mock-treated controls, treated or not with the RT inhibitor AZT, the CXCR4 antagonist AMD3100 and the IN strand transfer inhibitor Ral.
C. Percentage of HIV R5 using strain BaL replication in $LEDGF^{+/-}$ and $LEDGF^{-/-}$ KO mutants relative to wild-type and mock-treated controls, treated or not with the RT inhibitor AZT, the CXCR4 antagonist AMD3100 and the IN strand transfer inhibitor Ral.
WT, wild-type, MOCKtr, mock transfected, ND, no drug

5.-HIV INTEGRATION IN $LEDGF^{-/-}$ KNOCK-OUT CELLS

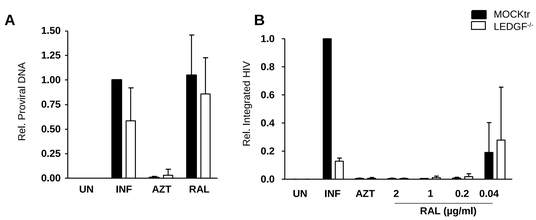


Figure 5. Integration of HIV NL43 is impaired in $LEDGF^{-/-}$ KO cells.
A. Proviral DNA formation in $LEDGF^{-/-}$ KO cells related to Mock-treated controls with or without the RT inhibitor AZT and the IN strand transfer inhibitor Ral respectively.
B. Viral integrated DNA in $LEDGF^{-/-}$ KO cells related to Mock-treated controls with or without the RT inhibitor AZT and increasing concentrations of the IN strand transfer inhibitor Ral respectively. Unless denoted, all treatments are supplemented 12h after infection with 10 µ g/mL of the anti-gp120 monoclonal antibody IgGb12 to avoid multiple replication cycles.
UN, uninfected , INF, infected, AZT, RT inhibitor AZT, RAL, IN strand transfer inhibitor

CONCLUSIONS

- Present study describes the generation of LEDGF/p75 knock-out cells using a ZFN that successfully recognizes and disrupts the sequence of the *PSIP1* gene coding for the C-terminal end of the LEDGF/p75 protein.
- The truncation of the C-terminal end of the LEDGF/p75 results in an impaired HIV-1 replication independent of genetic modification concerning the N-terminal functional domains or the Integrase Binding Domain (IBD) of LEGDF/p75 protein. Further studies must be carried out to elucidate the functional role of genetic variants in the non-coding regions of the *PSIP1* in vivo.
- Observed residual infection and HIV integration into the host genome in $LEDGF^{-/-}$ KO cells confirms previous data indicating that other pathways rather than LEDGF/p75 might allow HIV integration.
- The ZFN_{LEDGF} provides a new cellular model to study host factors involved in the HIV-1 integration process.