Silencing of HIV by a hairpin-loop DNA("siDNA")

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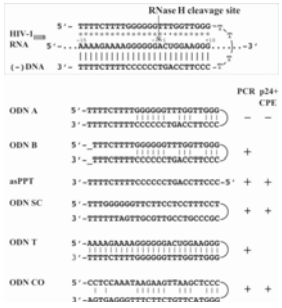
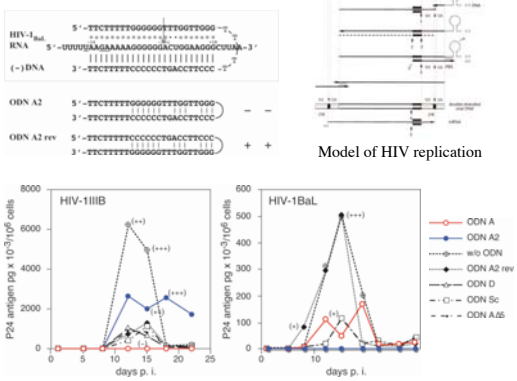
Abstract

We describe an RNA silencing by a hairpin-loop DNA, which inhibits HIV replication. A partially double-stranded 54mer DNA oligonucleotide (ODN) was targeted to the polypurine tract, PPT, of HIV. It inhibits virus replication including primary cells and drug-resistant primary HIV isolates (1,2). We demonstrate now that it prevents steps before DNA provirus formation. The effect of the ODN on HIV replication in cell culture is highly sequence-specific and sensitive to changes in length and single mismatches on either strand of the DNA (3). An ODN effective against HIV-IIIB was of low efficiency against HIV-BaL and vice versa, whereby their PPT's differ by two of 24 nucleotides. Thus, the structure and sequence of both strands of the ODN are important in cellular assays. The ODN leads to an RNA-DNA hybrid at the PPT, a structure, which induces cleavage of the viral RNA by the RT/RNase H in virus particles. For in vivo studies we developed the SFFV (Spleen Focus Forming Virus) as animal model of HIV. Treatment of mice with the ODN against SFFV reduced the virus titer. Furthermore, we quantified the individual contributions of Ago2, the cellular RNase H1, and the viral RT/RNase H to the ODN-mediated silencing of the viral RNA by knock-down of the individual components in T lymphocytes. We noticed that the RT/RNase H can silence HIV RNA also with siRNA even though less efficiently than with the ODN. Our data demonstrate that the structural relationship between PIWI and RNase H is supported at the enzymatic level with the viral RNase H. The ODN may be designated as siDNA.

(1,2) Jendis et al. AIDS Res. Hum. Retroviruses 23,1204 (1996) and 14, 999 (1998), (3) Moelling et al., FEBS Letters 580, 3545 (2006), (4) Moelling et al., *Cold Spring Harbor Symposia on Quantitative Biology, Vol. LXXI, CSHL Press 978-087969817-1* (2007), (5) Matskevich et al. AIDS Res Hum Retroviruses **22**, 12, 1220-1230 (2006).



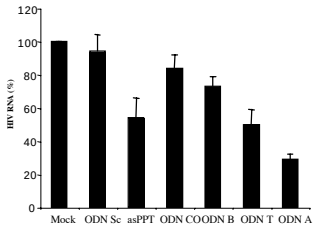
ODN A and variants targeting the PPT of HIV-1IIIB or HIV-1BaL



Sequence-specific effec of ODN A on HIV-1 IIIB and less on HIV-1 BaL

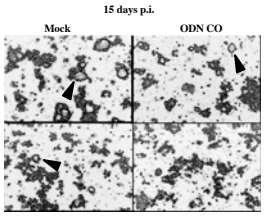
Model of HIV replication

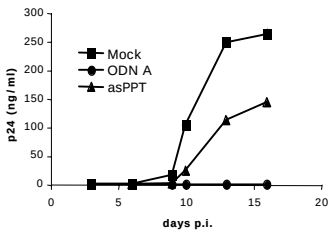
ODN A silencing of HIV- RNA in virions

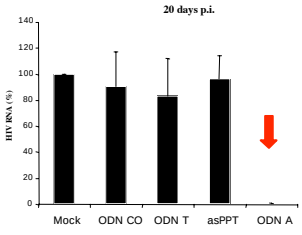


ODNs induce RT/RNaseH-dependent cleavage in virions in a sequence-specific manner

ODN A - treated virions (4h) lost infectivity in cell culture



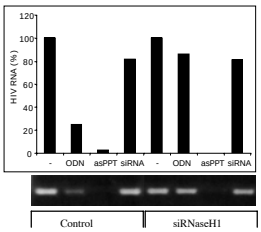




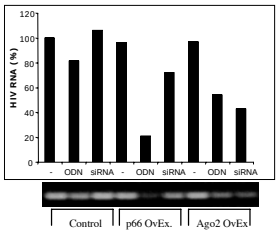
C81-66/45 cells were infected with virions preincubated with ODN A, ODN T, ODN CO or asPPT for 4h, at MOI of 0.01. RNA was extracted from infected cells and HIV replication was analysed by observing synsytia formation (15 days p.i.), by measuring p24, and by real-time PCR, 20 days p.i.; Syncytia formation (indicated by arrowheads) is observed in the cells infected with control ODN-treated and not ODN A-treated virions.

Lysate supplementation assay

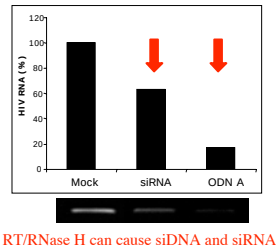
Knock-down of cellular RNase H1 shows, that RNase H1 contributes to ODN A-mediated cleavage of HIV RNA



Overexpression of HIV RT/RNase H (p66) and Ago2 increases ODN A-and siRNA-mediated cleavage of HIV RNA in RNaseH1-knochdown cells

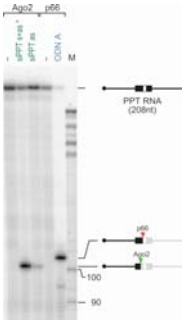


Recombinant HIV RT/RNaseH can mediate the cleavage of HIV RNA in the presence of siRNA

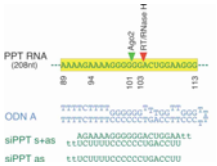


RT/RNase H can cause siDNA and siRNA mediated RNA silencing

Immunoprecipitated enzymes

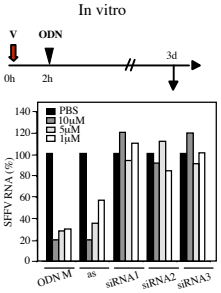


siRNA- and "siDNA" -mediated cleavage of PPT RNA by immunoprecipitated Ago2 or RT/RNase H (p66). * The ds siRNA was applied by transfection, all other oligonucleotides in vitro.

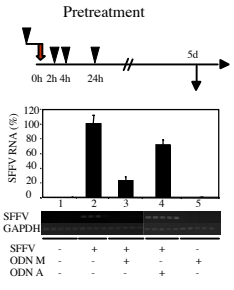


SFFV-mouse model

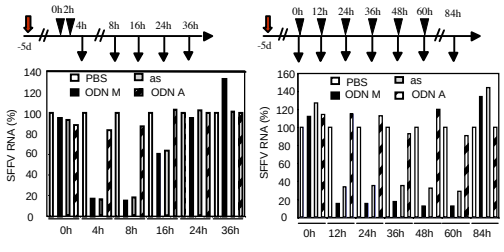
In vitro



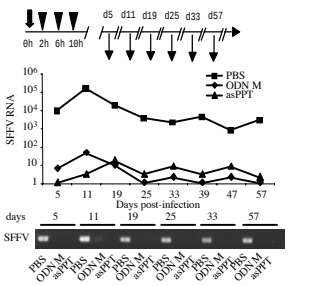
Pretreatment

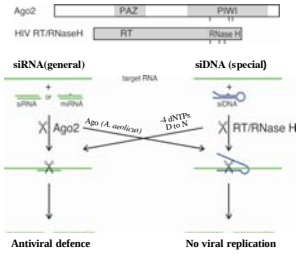


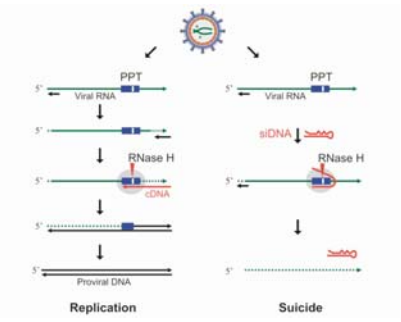
Short term kinetics



Abrogation of SFFV Infection







Conclusions

- ODN A induces "silencing" of HIV-RNA in vitro, in virions, and in cell culture.
- ODN M is effective in a SFFV-mouse model (survival, sterility).
- Viral RT/RNase H is the major effector of "siDNA".
- Viral RT/RNase H can also induce "silencing" of HIV-RNA by siRNA, RNase H related to Ago2.