Heterochromatin structure is induced by siRNA targeting HIV-1 promoter region

K Suzuki[1], H Lim[1,3], T Ishida[2], T Watanabe[2], D Cooper[1,3], A Kelleher[1,3]

1. Centre for Immunology, St Vincent's Hospital, Sydney, Australia
2. Medical Genome Sciences, Tokyo University, Japan
3. NCHECR, UNSW, Sydney, Australia

Kazuo Suzuki.
Centre for Immunology
St Vincent's Hospital
Victoria Street Darlinghurst
Sydney NSW 2010 Australia
Telephone +61-2-8382-2818
Facsimile +61-2-8382-2391
E-mail k.suzuki@cfi.unsw.edu.au

Introduction

- We previously reported that small interfering (si)RNA targeting the promoter region of HIV-1 can suppress viral replication at transcriptional level though Transcriptional Gene Silence (TGS) mechanism (Fig-1).
- Nuclear run-on assay data demonstrate that this suppress at transcriptional level.
- siRNAs induce RNA directed DNA methylation of cytosines within the HIV promoter.
- The methylation inhibitor, 5-aza-C, reverses this siRNA induced silencing.
- We sought evidence that this silencing was associated with chromatin remodeling.

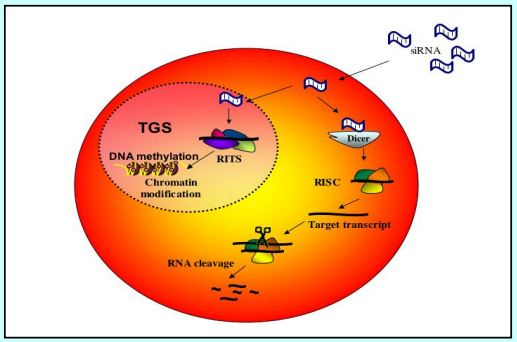

Fig.-1 siRNA can induce degradation of homologous mRNA though function of RISC and siRNA can also interact promoter DNA to induce DNA methylation and chromatin compaction, inhibiting initiation of gene transcription.

Aim

- To demonstrate that the siRNA targeting HIV-1 promoter can induce chromatin modification using Chromatin immunoprecipitation (ChiP) assay.
  - reclutement of K9 methylated histone (H3K9methy), and argonaute-1 (Ago-1) could be expected in HIV promoter region with siRNA suppressed culture

Method

- MAGIC-5 cells (CCR5 and CXCR4-expressed HeLa/CD4+ cells) were used for HIV-1 infection of molecualar clone of NL4-3 (Fig-4).
- Virus production was measured by reverse transciptase (RT) levels in supernatant.
- ChiP assays were determined reclutemnt of the specific Histone.

Results

Effect of six short siRNAs on productive HIV-1 infection including 4 siRNAs targeting HIV-1 promoter region and siRNA HIV-gag, which is known act as PTGS.

(A), The sequences within HIV-1 5'LTR targeted by *HIV-prom-A-D* siRNAs are indicated by highlighting. Nucleotide numbering is relative to the transcription start site and sequence is that of HIV-1 strain NL4-3. Each siRNA contains at least one CpG site. (B), Effect of siRNAs on the time course of HIV-1 (NL4-3) production in MAGIC-5 cells. At day 7 post-infection siRNAs were transfected into the cells (indicated by arrow). Reverse transcriptase (RT) levels are shown for cultures transfected with HIV-prom-A, B, C, D, HIV-1-gag, and scrambled siRNAs as indicated in the figure legend. (C), HIV-1 proviral DNA is present in cultures transfected with HIV-prom siRNAs regardless of whether infection is productive or not. PCR amplification of proviral DNA extracted from MAGIC-5 cells 38 days after infection. The amplified region is a 154bp fragment of the HIV-1 gag gene. NC indicates non-infected MAGIC-5 cells.(Fig-2).

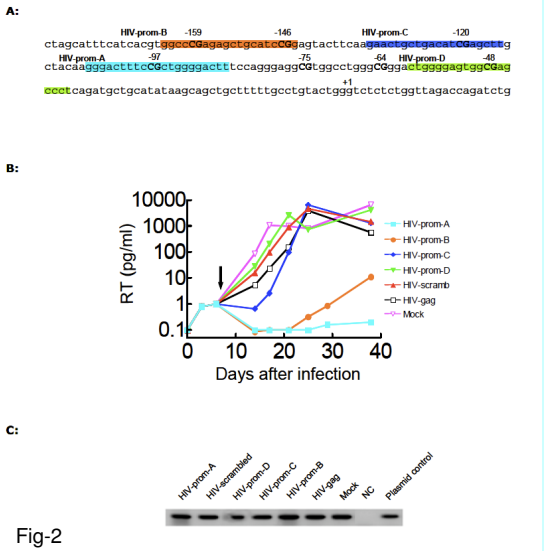

Fig-2

Enrichment of Ago1, H3K9me2, and HDAC1 associated with HIV-LTR in silenced siRNA-transfected cells (comparing Prom-A and Prom-D).

ChIP assays were conducted at days 1 and 11 post-transfection on extracts from formaldehyde-fixed MAGIC-5 cells treated with prom-A, prom-D, or mock transfection. DNA fragments from whole-cell extracts were co-precipitated with antibodies against the following: (*A),* Ago1; (*B),* H3K9me2, and (*C),* HDAC1, and then amplified by PCR. HIV-1-LTR copy numbers obtained from each immunoprecipitation were normalized against that obtained from whole-cell extracts. Each value shown is the relative enrichment of three separate experiments (mean S.E.) normalized to the value obtained from the mock transfection culture (Fig-3).

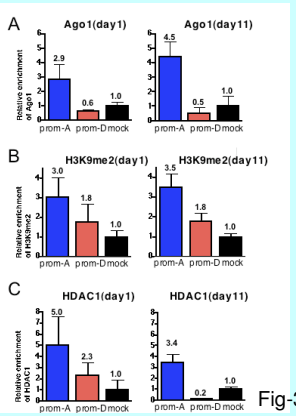

Fig-3

Enrichment of H3K9me2 associated with HIV-LTR and HIV-*gag* in prom-A siRNA-transfected cells indicates extensive regional heterochromatin formation.

ChIP assays were conducted day 11 post-transfection with antibodies against HDAC1 and H3K9me2 for the analysis of the following: (*A),* upstream Nuc-0 region; (*B),* downstream site within the coding region of *gag. (C),* model of heterochromatin formation induced by siRNA targeting HIV-1 promoter region. siRNA acts as a nucleation center for recruitment of the RNA-induced transcriptional silencing (RITS) complex and closed chromatin formation extends both upstream and downstream to include adjacent promoter and mRNA coding regions. siRNA (*purple line*) is loaded into Ago1. The RITS machinery, including histone deacetylase (*HDAC1*) and histone methyltransferase (*HMT*), induces H3K9me2 (*blue flag*) and recruits heterochromatin protein 1 (*HP1*) to the area surrounding the siRNA target site. Complexes including HP1 and HMT spread beyond the initial site of recruitment, creating broad domains of heterochromatin structure as indicated by H3K9me2 status of both the upstream promoter and downstream *gag* regions (Fig-4).

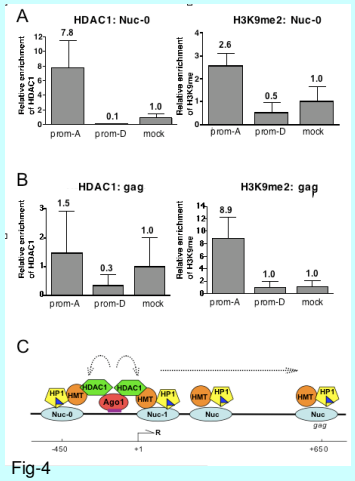

Fig-4

Short siRNAs targeting the U3 region of the HIV-1 promoter have limited PTGS activity.

(*A),* map of the HIV-1 3'-LTR under the control of the immediate early CMV promoter, with the location of sequences targeted by four previously described siRNAs and code-R siRNA. Code-R siRNA has sequence homologous to part of the R region of HIV-1 3'-LTR upstream of the polyadenylation sites. The position of PCR primers used for the detection of mRNA of HIV-LTR is indicated with *arrows*. (*B),* identification of two clones stably transfected with high expression of HIV-3'-LTR under the immediate early CMV promoter. RT-PCR was used to detect 3'-LTR expression, and two expressing clones are shown. One clone, CMV-3LTR1–4, with the highest level of expression of 3'-LTR messenger RNA, was chosen for further study. *(C),* assessment of the extent of gene silencing by PTGS following transfection of clone CMV-3LTR1–4, with each of four U3 region-targeted siRNAs or R region-targeted siRNA. LTR mRNA levels were assessed by real time RT-PCR 48 h after transfection. Real time PCR data are shown as a relative reduction in HIV-mRNA levels normalized to the value obtained from mock transfection experiments. Results shown are from three independent experiments (mean S.E.) (Fig-5).

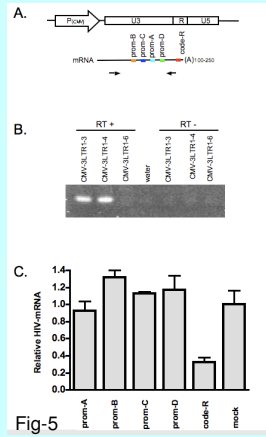

Fig-5

Conclusion

- siRNA targeting HIV promoter region induces HIV gene silence.
- Reclutement of K9 methylated histone-3, Ago-1, and HDAC1 in HIV promoter region with siRNA suppressed culture.
- Elevated levels of H3K9me2 and HDAC1 spread upstream of the target sequence, and elevated H3K9me2 levels also spread downstream into the coding region.
- Although there is a theoretical possibility that the observed viral suppression could be mediated by the PTGS mechanism with this siRNA acting at the 3'LTR of the virus, we demonstrate that prom-A siRNA, and three other U3 targeted siRNAs, are inefficient inducers of PTGS.
- **These induced changes of elevated HDAC1 and H3K9me2 in prom-A siRNA suppressed culture are consistent with those described in latent HIV-1 infection.**

Acknowledgement

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