The HIV Capsid-Binding Host Factor CPSF6 is Post-Transcriptionally Regulated by the Cellular Micro-RNA miR-125b

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ABSTRACT

Cleavage and polyadenylation specificity factor 6 (CPSF6) is a cellular protein involved in miRNA processing. Emerging evidence suggests that CPSF6 also plays key roles in HIV-1 infection, specifically during nuclear import and integration target-1. However, the cellular and molecular mechanisms that regulate CPSF6 expression are largely unknown. In this study, we report a post-transcriptional mechanism that regulates CPSF6 via the cellular microRNA miR125b. An in silico analysis revealed that the 3’ untranslated region (3’UTR) of CPSF6 contains a miR-125b-binding site that is conserved across several mammalian species. Since miRNAs repress protein expression, we tested the effects of miR-125b expression on CPSF6 levels in miR-125b knockout and over-expression experiments, revealing that miR-125b and CPSF6 levels are inversely correlated. To determine whether miR-125b post-transcriptionally regulates CPSF6, we introduced the 3’UTR of CPSF6 miRNA into a luciferase reporter and found that miR-125b negatively regulates CPSF6 3’UTR-driven luciferase activity. Accordingly, mutations in the miR-125b seed sequence abrogated the regulatory effect of the miRNA on the CPSF6 3’UTR. Finally, pull-down experiments demonstrated that miR-125b physically interacts with CPSF6 3’UTR. Interestingly, HIV-1 infection downregulated miR-125b expression concurrent with upregulation of CPSF6. Notably, miR-125b downregulation in infected cells was not due to reduced pri-miRNA or pre-miRNA levels. However, miR-125b down-regulation depended on HIV-1 reverse transcription but not viral DNA integration. These findings establish a post-transcriptional mechanism that controls CPSF6 expression and highlight a novel function of miR125b during HIV-host interaction.

RATIONAL

Figure 1. CPSF6 3’UTR harbors a binding site for miR-125b
(A) In silico analysis predicted strong binding between CPSF6 and miR-125b.
(B) RNA BiFold Analysis
(C) miR-125b binding sequence in CPSF6 3’UTR is conserved across multiple species.

Figure 2. CPSF6 Protein Expression Regulated by Transcription of miR-125b mimics/anti-miRs.
(AC) 293T cells (D-H) SUPT1 T-cells have endogenously higher miR-125b levels than CEM. miR-125b transfections were applied accordingly. (I-J) THP1 monocytes were previously shown to have CPSF6 regulated effects of HIV-1 inhibition.

Figure 3. CPSF6 3’UTR Activity is Regulated by miR-125b.
(A) Three constructs were developed to probe the effect of miR-125b on the 3’UTR of CPSF6.
(B) Constructs transfected into 293T cells. (C-F) Constructs co-transfected with miR-125b mimic/anti-miRs.

Figure 4. Mutations in the miR binding sequence abrogate the regulatory effects of miR-125b on the CPSF6 3’UTR.
(A-C) RNA BiFold analysis of CPSF6 3’UTR mutant sequences shows altered binding. (D-E) Relative luciferase expression of endogenous CPSF6 3’UTR compared to two mutant 3’UTRs. (E-G) Relative luciferase expression of endogenous vs mutant CPSF6 3’UTR with co-transfection of miR mimic/anti-miRs. (H-I) miR-125b physically interacts with CPSF6 3’UTR complexes were pulldown with streptavidin-coated magnetic beads. Enrichments of miRNAs of interest were quantified by qPCR with primers complementary to their respective 3’UTRs. Data shown are enrichment of PAPR-1 (positive control), actin (negative control) and CPSF6 3’UTR levels. Blue bars represent pulldowns with miR-mimics whereas red bars represent pulldowns with scrambled controls.

Figure 5. miR-125b physically interacts with CPSF6 mRNA. HEK-293T cells were transfected with biotinylated-miR-125b mimics or scrambled controls and the miR-125b/miRNA complexes were pulled down with streptavidin-coated magnetic beads. Enrichments of miRNAs of interest were quantified by qPCR with primers complementary to their respective 3’UTRs. Data shown are enrichment of PAPR-1 (positive control), actin (negative control) and CPSF6 3’UTR levels. Blue bars represent pulldowns with miR-mimics whereas red bars represent pulldowns with scrambled controls.

Figure 6. HIV-1 infection downregulates miR-125b. (A-D) miR-125b expression in HIV-1 infected 293T cells. (E-F) miR-125b expression in HIV-1 infected SUPT1 cells. (G-I) CPSF6 expression of corresponding 293T (left) and SUPT1 (right) infected cell lysates.

Figure 7. miR-125 infection does not affect miR precursors. (A-D) pri-miR-125 was biotinylated and cotransfected with biotinylated miR-125 or pre-miR-125 into HEK-293T cells. Enrichments of miRNAs of interest were quantified by qPCR with primers complementary to their respective 3’UTRs. Data shown are enrichment of PAPR-1 (positive control), actin (negative control) and CPSF6 3’UTR levels. Blue bar represents pulldowns with miR-mimics whereas red bar represents pulldowns with scrambled controls.

RESULTS

HYPOTHESIS

Anti-HIV microRNA miR-125b Directly Targets CPSF6 3’UTR to Regulate Expression

BACKGROUND

- Binding to CD4 and co-receptor
- Reverse Transcription of Viral RNA
- Nuclear Import of Pre-Integration Complex
- Integration of Viral DNA into Host Genome

> The Preintegration Complex (PIC), containing the viral DNA and other viral and cellular proteins, is actively transported into the nucleus of the target cell through the nuclear pore complex (NPC) CPSF6 Plays a Key Role in Integration Targeting

- A variety of cellular proteins have been implicated in PIC nuclear import
- Many of the cellular factors involved in PIC nuclear import also facilitate targeting of the viral DNA integration into gene dense chromosomal regions.
- Recent studies have clarified that CPSF6 is a key player in targeting HIV-1 DNA into the gene dense region of the host chromosomes

CONCLUSIONS AND FUTURE DIRECTIONS

- CPSF6 is a direct target of miR-125b
- Revealed activation of miR-125b mediated regulation of CPSF6 to promote HIV-1 nuclear entry
- Elucidate potential role of DNA Methylation regulation on miR-125b biogenesis

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