miRNA-103 MODULATES CCR5 AND AFFECTS ESTABLISHMENT OF HIV-1 LATENCY IN CD4+ T CELLS

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BACKGROUND

- MiRNAs are small regulatory RNAs which affect the stability or the translation of target mRNAs
- We recently identified the p53-regulated miRNA-103 as a modulator of CCR5 expression in macrophages(a)
- It has been shown that activated-to-memory transitioning CD4+ T cells upregulate CCR5, the HIV-1 co-receptor, and are more susceptible to latent HIV-1 infection(b)
- ***Hypothesis : By contributing to the control of CCR5 expression in CD4+ T lymphocytes, miRNA-103 could play a role in the establishment of latent HIV-1 reservoirs in vivo***

TAKE HOME MESSAGE

- Pharmacologic stabilization of p53 in CD4+ T cells **decreases CCR5-dependent HIV-1 infection** via upregulation of miRNA-103, reducing the frequency of productively and latently infected cells
- Reduced CCR5 mRNA levels in CD4+ T cells of elite and viremic controllers **correlates** with an upward miRNA-103 expression

METHODS

- Participants details :

	Elite controllers (EC)	Viremic controllers (VC)	ART-naïve progressors
Number of participants	9	9	5
Number of males/females	6/3	5/4	5/0
Age in years*,#	50 (35-60)	40 (26-59)	31 (24 - 59)
Duration of infection (year)*,#	13 (7 - 20)	3 (1 - 24)	< 0.5
CD4 counts (cells/µL of blood)*,#	700 (310 - 1080)	610 (420 - 1040)	550 (510 - 700)
CD8 counts (cells/µl of blood)*,#	730 (260 - 1320)	760 (490 - 1590)	1460 (1310 - 3832)
Viral loads (copies/mL of plasma)*,#	< 50	714 (70 - 2777)	152 703 (34 276 - 248 677)
Years of undetectable viral load (EC) or <3000 copies/ mL (VC)*,#	8 (5 - 15)	2 (1 - 17)	N/A

* Median with range # At sample acquisition N/A = Not applicable

- Statistical analyses : Non-parametric Kruskal Wallis tests were used to compare ranks between more than two groups. Non-parametric Mann-Whitney's U-tests (two-tailed) were used to compare ranks between two groups. A *P* value of less than 0.05 was considered statistically significant. *, **, ***, **** signify <0.05, <0.01, <0.001, <0.0001, respectively; ns, not significant.

RESULTS

CCR5 is regulated by miRNA-103 in CD4+ T cells

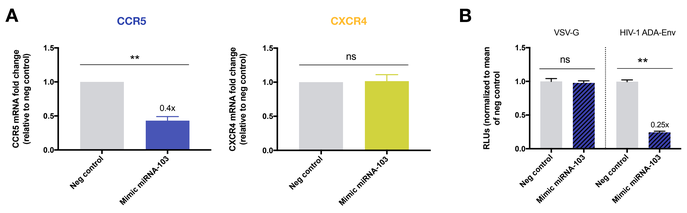


Figure 1 - MiRNA-103 downregulates CCR5 mRNA levels in the C10/MJ lymphocyte cell line. (A) CCR5 and CXCR4 mRNA were measured in miRNA-103 mimic or control-transfected C10/MJ cells. (B) Luciferase activity was measured in lysates of either NL4-3-Luc+Vpr+ -ADA-Env or -VSV-G infected C10/MJ cells previously transfected with controls or miRNA-103 mimics.

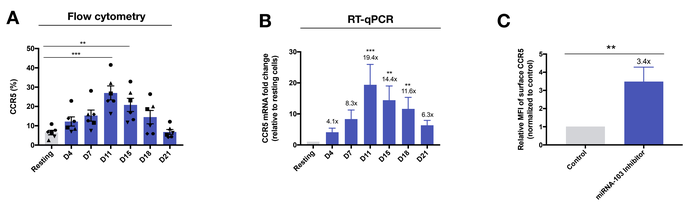


Figure 2 - MiRNA-103 modulates CCR5 expression in primary CD4+ T cells transitioning to a resting phenotype. (A and B) HIV-1 co-receptor CCR5 expression was measured by flow cytometry/RT-qPCR and compared in resting (D0), activated (D4) and relaxed CD4+ T cells (D7-D21) obtained from healthy donors. (C) Primary CD4+ T cells (D11) were transfected with FAM-labeled miRNA-103 inhibitors. Mean fluorescence intensity (MFI) of CCR5 protein at the cell surface was measured and compared to control transfected cells.

MiRNA-103 is modulated by the p53 pathway in primary CD4+ T cells

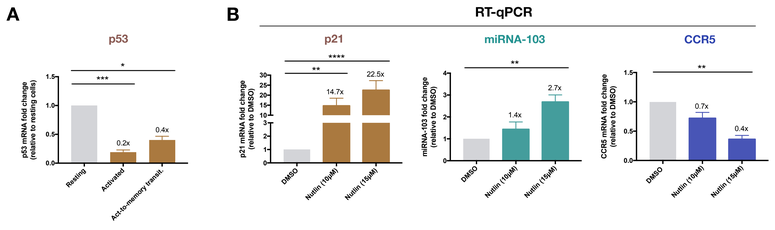


Figure 3 – Role of p53 on miRNA-103 and CCR5 expression levels in primary CD4+ T cells. (A) p53 mRNA was measured by RT-qPCR and compared in resting (day 0), activated (day 4) and activated-to-memory (day 11) CD4+ T cells. Data are pooled from six blood donors. (B) Different concentrations of the MDM2 antagonist Nutlin-3 were added to activated primary CD4+ T cells and the effect on p21 mRNA, miRNA-103 and CCR5 mRNA were determined by RT-qPCR. Data are pooled from five blood donors.

Nutlin-3 decreases the susceptibility of CD4+ T cells to HIV-1 latent infection

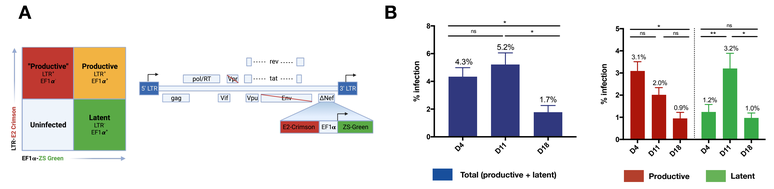


Figure 4 - Activated-to-memory transitioning CD4+ T cells are more susceptible to latent infection. A) Schematic representation of the HI.fate.E dual fluorescent HIV-1 reporter vector (B) Activated primary CD4+ T cells were infected with HI.fate.E-JRFL-Env viruses at different time points post-activation and E2-Crimson and ZS-Green expression analyzed by flow cytometry two days after infection.

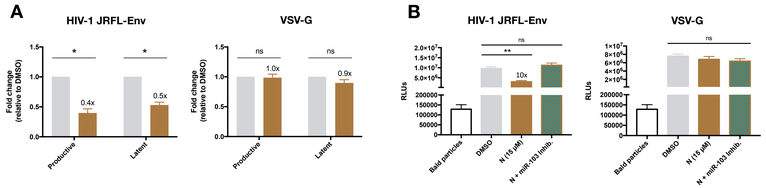


Figure 5 - Nutlin-3 treatment decreases the susceptibility of transitioning CD4+ T cells to HIV-1 infection. (A) Primary CD4+ T cells were activated for 4 days, treated with DMSO or Nutlin-3 (15µM) for 24h, then infected with the indicated HI.fate.E viruses (HIV-1 JRFL-Env or VSV-G pseudotyped) for two days, and the percentage of productive/latent infected cells determined and normalized to DMSO. (B) Primary CD4+ T cells were activated for 4 days and transfected with miRNA-103 inhibitor or controls, treated with vehicle or Nutlin-3 (N) at 15µM for 24h, and then infected with NL4.3-NanoLuc+ viruses pseudotyped with HIV-1 JRFL-Env or VSV-G. Bald particles (without any viral glycoproteins) were also used as a control. Nano-Luciferase activity was measured in cell lysates after 48h. Data are pooled from four different blood donors.

CCR5 is downregulated in CD4+ T cells of EC/VC individuals

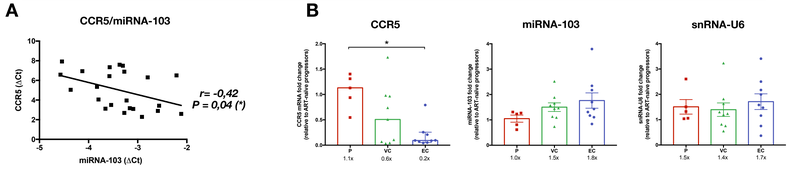


Figure 6 – Analyses of miRNA-103 and CCR5 mRNA expression levels in CD4+ T cells isolated from HIV-1-infected individuals. (A) CD4+ T cells from ART-naïve progressors (P), elite controllers (EC) and viremic controllers (VC) were isolated from PBMCs by negative selection. Following total RNA extraction, miRNA-103 and CCR5 mRNA levels were measured by RT-qPCR. Based on ΔCt values, a negative correlation between miRNA-103 expression and CCR5 mRNA levels in CD4+ T cells from all groups was determined by Pearson r test, values: *p < 0.05. (B) Relative expression (fold change) of miRNA-103, snRNA-U6 and CCR5 mRNA in CD4+ T lymphocytes from EC (n=9), VC (n=9) and ART-naïve progressors (n=5) were determined by RT-qPCR, and compared to that of the mean of ART-naïve progressors.

CONCLUSION

- Activated-to-memory transitioning cells express high levels of CCR5 and have a cellular environment conducive to latent infection.
- The p53-regulated miRNA-103 targets CCR5 mRNA in CD4+ T cells and participates in its regulation.
- We observed a decrease of CCR5 mRNA in CD4+ T cells from EC/VC individuals as well as an upward trend of miRNA-103 in these cells, suggesting that miRNA-103 contributes in part to the control of CCR5 expression in the CD4+ T cells.

ADDITIONAL INFORMATION

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- References : (a)Lodge & al. 2020, mBio ; (b)Shan & al. 2017, Immunity