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Expression of MDM2 in macrophages favors HIV-1 integration through inhibition of p53.

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Background

Macrophages play an important role in the establishment and propagation of HIV-1 infection. Upon exposure to HIV-1, only a small proportion of macrophages are infected whereas most remain uninfected.
To shed light on this issue, transcriptomic and proteomic analyses were performed to compare the virus-infected and uninfected bystander populations and determine the molecular basis of HIV-1 permissiveness in macrophages. Precise mapping of the interactions between HIV-1 and host signaling pathways identified several new potential host-encoded regulators of HIV-1 replication in macrophages.
One of these was MDM2, an E3 ubiquitin ligase regulating the stability of various proteins, one of them being p53.

Material and Methods

Flow Cytometry

Monocyte-derived macrophages (MDMs) were transfected with siRNA or treated with chemical inhibitors, then exposed to a fully replicative R5-tropic virus coexpressing the murine Heat Stable Antigen (HSA). Infection was monitored by flow cytometry after 3 days, detecting cells expressing HSA at their surface.



Figure 1: Schematic representation of the NL4-3-Bal-IRES-HSA
Heat Stable Antigen (HSA), also known as murine CD24 antigen, is transcriptionally fused to Nef through an IRES sequence into HIV-1 NL4-3 molecular clone in which the wild type envelope was replaced by that of the Bal isolate.

qRT-PCR

MDMs were transfected or treated with chemical inhibitors, then exposed to a fully replicative R5-tropic virus. Efavirenz was added after 24h to ensure a single round of infection. DNA was extracted after 2 days of infection and assessed by qRT-PCR for the number of completed transcripts, 2-LTR circles and integrated HIV-1 genomic DNA copies (Alu-HIV-1 PCR).

References

1. **Imbeault**, M., Lodge, R., Ouellet, M. & Tremblay, M. J. (2009) Virology 393, 160–167.
2. **Dornadula** G., Zhang H., Shetty S., Pomerantz R.J. (1999) Virology 253, 10–16

Results

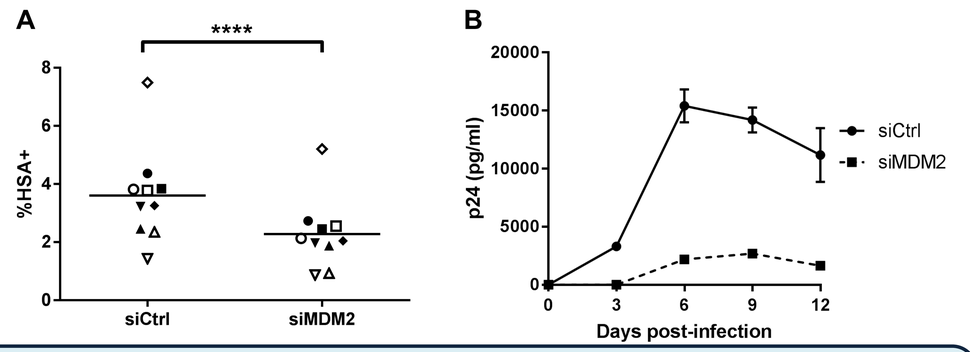

Figure 2: MDM2 gene silencing in macrophages decreases their susceptibility to HIV-1 infection
A) MDM2 silencing induced a 40% decrease in the percentage of HSA+ cells.
(ratio paired t-test, ****p < 0.0001, n=10)
B) p24 production was monitored by ELISA following exposition of MDMs to NL4-3-Bal. MDM2 knockdown decreased the production of *de novo* virions.

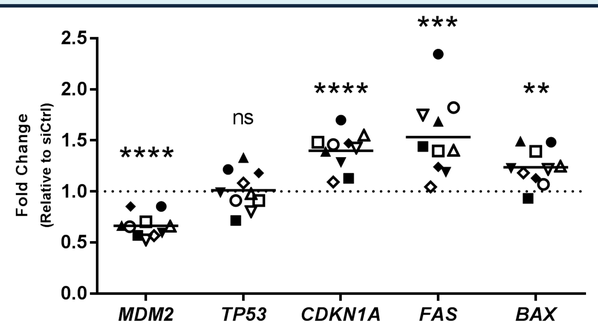

Figure 3: Gene silencing of MDM2 increase the mRNA level of known p53-induced genes
MDMs from 10 donors were transfected with siRNA against MDM2 for 48h, which corresponds to the moment of infection, then total RNA was extracted and assessed by qRT-PCR for p53-dependant genes.
- siRNA targeting MDM2 reduced efficiently its mRNA level.
- p53 mRNA level was not affected by MDM2 knockdown.
- MDM2 silencing increased p21, Fas and Bax mRNA level, suggesting an activation of p53.

(paired t-test, ns: non significant; **p < 0.01; ***p < 0.001; ****p < 0.0001, n=10)

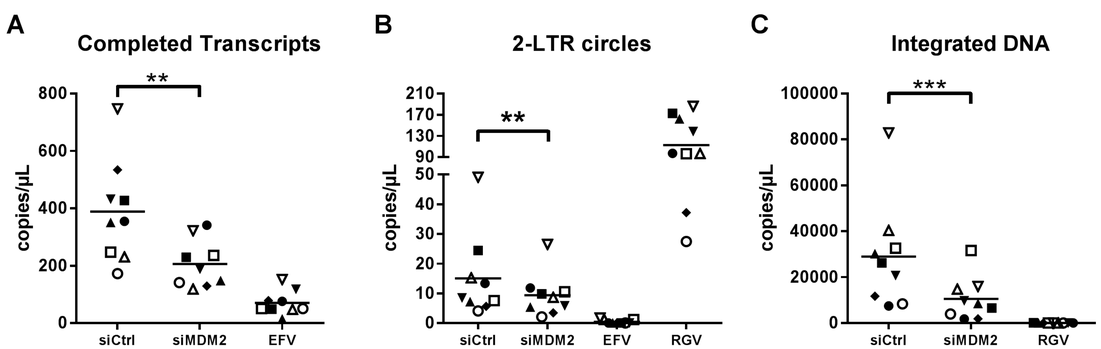

Figure 4: Gene silencing of MDM2 inhibits the number of integrated viral DNA in macrophages
MDMs from 9 donors were infected with NL4-3-Bal-IRES-HSA following transfection. DNA was extracted at 2 dpi. Efavirenz (EFV) and Raltegravir (RGV) were used as controls. Samples were assessed by qRT-PCR for the number of (A) completed transcripts, (B) 2-LTR circles or (C) integrated genomic DNA. MDM2 knockdown inhibited the steps leading to HIV-1 integration.
(ratio paired t-test, **p < 0.01; ***<0.001, n=9)

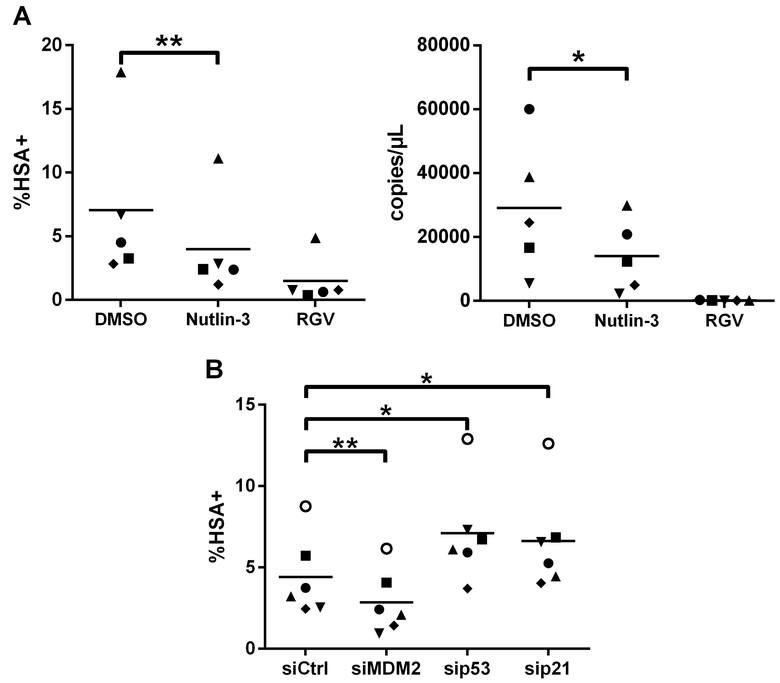

Figure 5: Activation of p53 reduces the susceptibility of MDMs to HIV-1 infection
A) MDMs were infected with NL4-3-Bal-IRES-HSA in presence of Nutlin-3 (500 nM). Infection was analyzed by flow cytometry after 3 days of infection (left) or 2 days of infection for the number of integrated copies (right).
(ratio paired t-test, *p<0.05; **p < 0.01; n=5)
B) Infection following the knockdown of p53 and p21 using specific siRNA increased the susceptibility of MDMs to HIV-1 infection.
(One-way ANOVA, *p < 0.05; **p < 0.01; n=6)

Conclusions

Our results indicate that MDM2 silencing induces p53-related genes expression and leads to a decrease in the number of integrated viral DNA copies. Stabilization of p53 using a chemical inhibitor also causes a resistance to HIV-1 infection. The expression level of MDM2 and p53 are therefore important factors in the establishment of the infection in MDMs. Identification of viral cofactors regulated by MDM2 will bring a new understanding of signaling events controlling HIV-1 replication in macrophages and reservoirs formation.

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