Investigating the Requirement of a NOD-like Receptor for Early HIV Replication

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Abstract

Human Immunodeficiency viruses rely on host-encoded proteins to facilitate their replication. A previous genome-wide siRNA screening approach (Koenig et al., 2008) resulted in the identification of NLRX1 as a host cellular protein required for early stages of HIV-1 replication. NLRX1 is a member of the Nod-like Receptor family, which collectively play critical roles in innate immune responses. Thus, our data provide a compelling link between an innate immune signaling receptor, NLRX1, and HIV-1 replication.

Methodology: HIV-1 luciferase reporter viruses pseudotyped by VSV-G and HIV-1 replication competent WT viruses were used to investigate the effect of silencing of NLRX1 on replication efficiency in various cell lines and primary cells. In addition, we assessed the infectivity levels of pseudotyped particles with amphotropic envelope using a pH-independant entry route and mapped the stage of viral inhibiton by real-time quantitative PCR. Effects on IRF3-dependant target gene expression were assessed by quantitative PCR and IFN ELISA.

Results: We first validated NLRX1 as a factor required for early-stage HIV infection in various cell lines, including 293T, HeLa and more physiological relevant cells, THP1, Jurkat cells and primary macrophages. NLRX1-inhibition resulted in a failure to initiate reverse transcription and was independent of the viral entry route. NLRX1 was recently reported to act as a negative regulator of mitochondrial antiviral immunity, through binding and inhibition of MAVS, an essential mediator of the RIG-I and IRF3 antiviral response. Based on the hypothesis that the inhibition of NLRX1 may render the innate surveillance machinery competent to respond to HIV infection, we sought to determine the effects of NRLX1 depletion upon IRF3 target gene transcription. We observed that ISG54, IFN-β and IL-6 was robustly induced upon infection with HIV in NLRX1-depleted cells, but importantly, NLRX1 silencing did not induce these genes in the absence of HIV.

1. NLRX1 - introduction

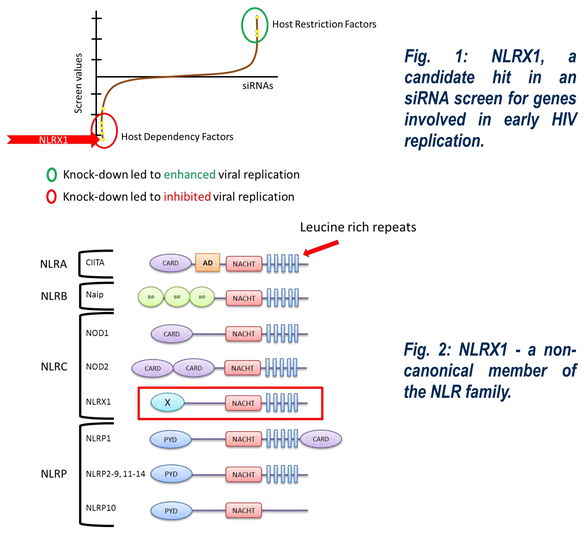


Fig. 1: NLRX1, a candidate hit in an siRNA screen for genes involved in early HIV replication.

Fig. 2: NLRX1 - a non-canonical member of the NLR family.

2. Confirming a role for NLRX1, independent of the viral entry route

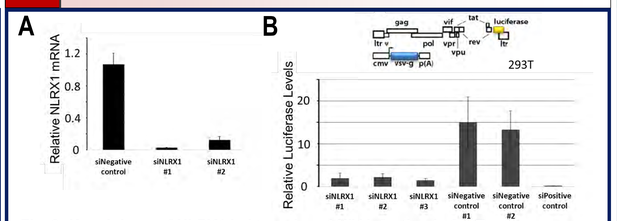


Fig. 3: Knockdown of NLRX1 led to reduced HIV infection in 293T cells.

NLRX1 was silenced by independent siRNAs and the cells were subsequently infected with a VSV-g pseudotyped single-cycle luciferase reporter virus. Knockdown efficiency (A) was determined and relative levels of luciferase (B) was assessed.

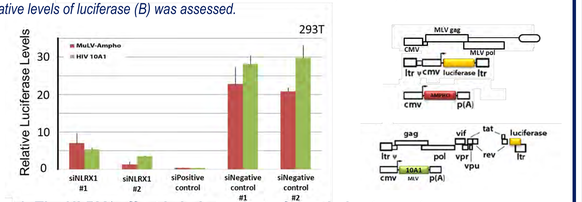


Fig. 4: The NLRX1 effect is independent of the viral entry route.

NLRX1 silenced in 293T cells were infected with viral vectors displaying pH-independent envelopes..

3. Mapping the stage of viral inhibition

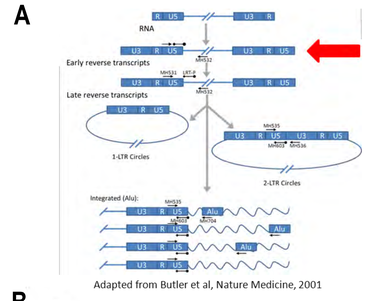
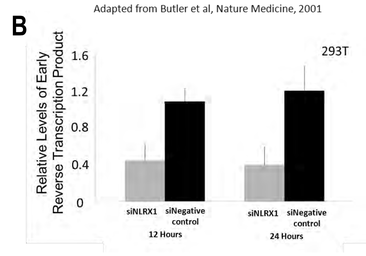


Fig. 5:

A) Schematic of PCR to determine different steps of the viral life cycle.



B) NLRX1 silencing leads to a viral block at early reverse transcription.

4. NLRX1 - a required host factor in T cells

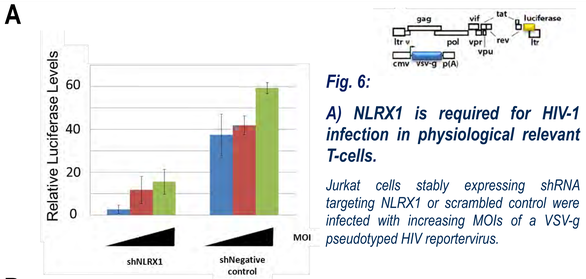
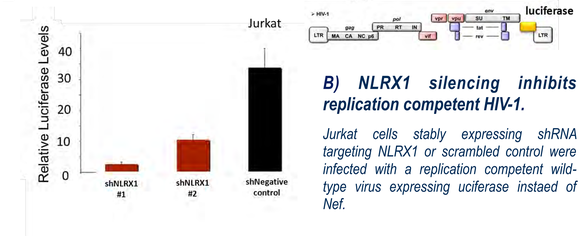


Fig. 6:

A) NLRX1 is required for HIV-1 infection in physiological relevant T-cells.

Jurkat cells stably expressing shRNA targeting NLRX1 or scrambled control were infected with increasing MOIs of a VSV-g pseudotyped HIV reportervirus.



B) NLRX1 silencing inhibits replication competent HIV-1.

Jurkat cells stably expressing shRNA targeting NLRX1 or scrambled control were infected with a replication competent wild-type virus expressing uciferase instaed of Nef.

5. NLRX1 - a required host factor in immune-competent myeloid cells

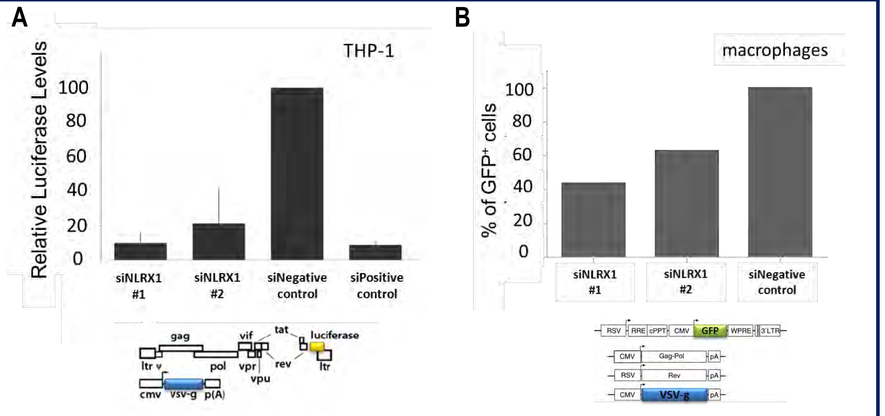


Fig. 7: NLRX1 silencing in innate immune-competent myeloid cells leads to decreased viral infection.

NLRX1 was silenced in THP-1 (A) and primary macrophages (B). The cells were subsequently infected with VSV-g prseudotyped HIV-1 reportervirus (A) or VSV-g pseudotyped lentiviral vector. (B).

6. NLRX1 silencing enables an innate immune response upon HIV infection

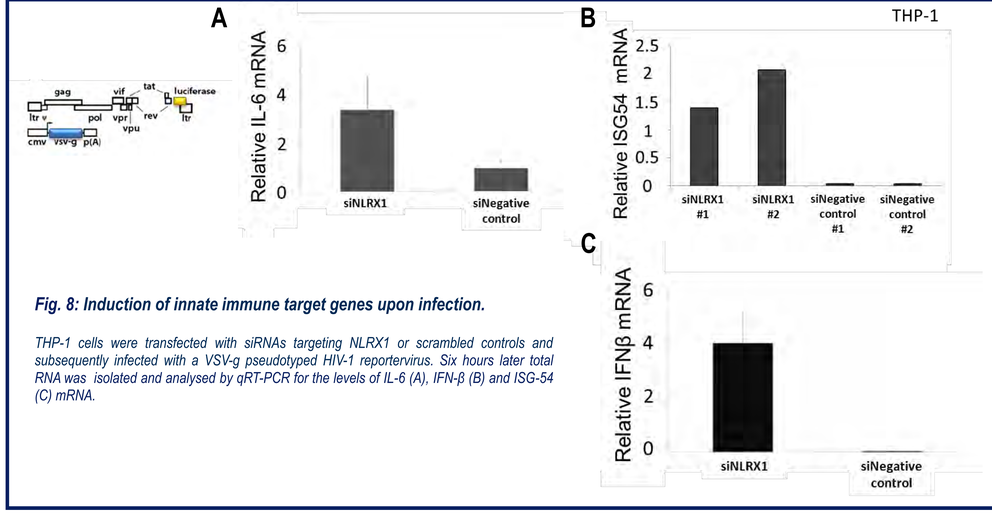


Fig. 8: Induction of innate immune target genes upon infection.

THP-1 cells were transfected with siRNAs targeting NLRX1 or scrambled controls and subsequently infected with a VSV-g pseudotyped HIV-1 reportervirus. Six hours later total RNA was isolated and analysed by qRT-PCR for the levels of IL-6 (A), IFN-β (B) and ISG-54 (C) mRNA.

Conclusion & Outlook

Conclusion:

These data suggest that restriction of HIV is not due to a generalized and persistent hyper-activation of an innate immune state in NLRX1-depleted cells. In conclusion, we demonstrate the requirement of NLRX1 for early HIV-1 replication.

Outlook:

We are currently validating the observed NLRX1 effect in various primary cell models (MDMs and MDDCs) and are investigating the molecular basis of NLRX1 dependency in early stage HIV replication.

Literature

Koenig et al, Cell, 2008; Fritz et al, Nature Reviews Immunology, 2006; Kanneganti, Nature Reviews Immunology, 2010; Ting et al, Science, 2010; Shaw et al, Current Opinion in Immunology, 2008; Butler et al, Nature Medicine, 2001