p21 regulates the HIV-1 restriction factor SAMHD1

Alba Ruiz[1], Eduardo Pauls[1], Eva Riveira-Muñoz[1], Marc Permanyer[1], Roger Badia[1], Bonaventura Clotet[1], Oliver T. Keppler[2], Ester Ballana[1], and Jose A. Este[1]

[1] IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain. [2] Institute of Medical Virology, University of Frankfurt, Germany

ABSTRACT

Background: CDK/INA (p21) is a cyclin-dependent kinase (CDK) inhibitor controlling cell cycle progression through binding and activation of cyclin-CDK1 or -CDK2 complexes. p21 expression has been known to restrict HIV-1 replication in macrophages by controlling the expression of the RNR2 subunit of the ribonucleotide reductase that, in turn, controls the intracellular deoxynucleotide (dNTP) pool required for HIV reverse transcription. dNTP levels are also tightly controlled by the dNTP triphosphohydrolase SAMHD1. We have evaluated the role of p21 in SAMHD1-mediated restriction of HIV infection.

Methodology: RNA interference was used to evaluate the role of p21, CDKs and SAMHD1 function in M-CSF monocyte derived macrophages (MDM). Protein expression was evaluated by mRNA qPCR or by Western Blot of the corresponding lysate. SAMHD1 phosphorylation at position T592 was used as a marker of SAMHD1 activation. HIV-1 infection was monitored by total proviral DNA or GFP expression of a pseudotyped expressing the VSV envelope and the GFP gene in the presence or absence of the SIVmac Vpx. TAED measurement of Ki67+ cells was used as a marker of cell proliferation.

Results: Monocyte stimulation with M-CSF leads to differentiation into macrophages and cell proliferation. RNAi of p21 expression led to an increase in the number of Ki67+ cells. Susceptibility to HIV-1 replication correlated to the degree of SAMHD1 inactivation as measured by SAMHD1 phosphorylation at T592. Delivery of SIVmac Vpx induced SAMHD1 degradation and subsequently increased virus infection. Under these conditions, siRNA-induced downregulation of p21 strongly enhanced the phosphorylation of SAMHD1 followed by an increase in HIV-1 proviral DNA formation and virus infection without affecting the overall SAMHD1 expression. The increased HIV-1 replication observed after SAMHD1 degradation was not affected or further enhanced by downregulation of p21, suggesting that the effect of p21 was tested dependent on SAMHD1 expression. SAMHD1 activity in primary cells (MDM and CD4+ T lymphocytes) was dependent on CDK2 as specific pharmacological inhibition or siRNA of CDK2 blocked SAMHD1 phosphorylation, proviral DNA and HIV-1 replication.

SAMHD1 is PHOSPHORYLATED in MONOCYTE-DERIVED MACROPHAGES (MDM) THAT ARE SUSCEPTIBLE TO HIV-1 INFECTION

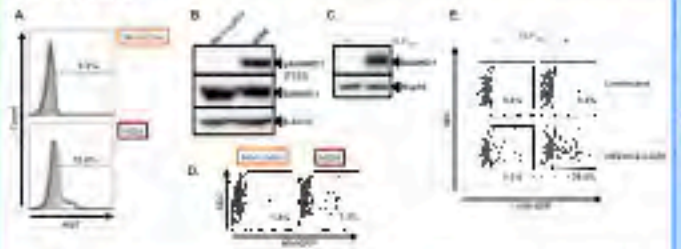


Figure 1. (A) Ki67 expression in monocytes from healthy (normoglycemic) control donors with after stimulation with M-CSF. (B) Lysates of monocytes and differentiated MDM were run in a polyacrylamide gel and blotted with an antibody recognizing the phosphorylated form of SAMHD1 at position T592, and SAMHD1 and anti-β-actin antibody. (C) SAMHD1 expression in MDM treated (+) or not (-) with viral-like particles carrying Vpx (VLPs$_{vpx}$) was analyzed by Western blot. Hsp90 antibody was used as a loading control. (D) Monocytes before and after 5-d stimulation with M-CSF were infected with a GFP-expressing, VSV-pseudotyped NL4-3 HIV-1 virus. Infection is shown measured by GFP+ cells 2-d post infection. (E) HIV-1 infection measured as percentage of GFP+ cells of macrophages in the absence (-) or presence (+) of receptor positive clinical isolate (VLP$_{vpx}$).

RNA INTERFERENCE of CDK2 and CDK6 INHIBITS SAMHD1 PHOSPHORYLATION

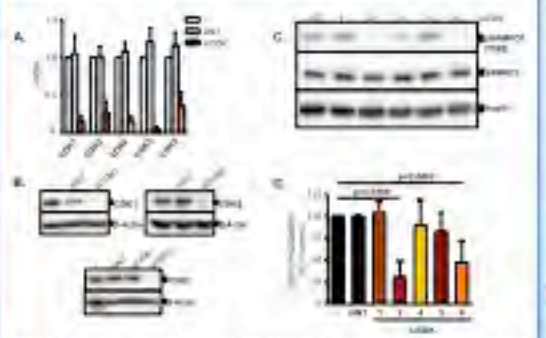


Figure 2. (A) siRNA of CDK1, 2, 4, 5 and 6 were quantified in samples of macrophages treated with siRNA targeting each of the CDK and normalized to samples transfected without siRNA (-). Macrophages treated with a non-targeting siRNA (siNT) were also included. (B) Lysates of the siRNA-treated macrophages were subjected to SDS-PAGE and blotted with an anti-CDK1, anti-CDK2, anti-CDK6 or anti-β-actin antibodies. (C) Lysates of macrophages treated with siRNA targeting CDK (siCDK) a non-targeting siRNA (siNT) or mock-transfected (-) were starved, transferred and treated with the phospho-specific Thr592 antibody (pSAMHD1(THR)) and SAMHD1 and Hsp90 antibodies. (D) Lysates were subjected to overnight immunoprecipitation of SAMHD1 followed by blotting with anti-phosphotyrosine followed by protein antibody (pTyr-Pro) and SAMHD1 antibody. Quantification of SAMHD1 phosphorylation relative to the mock-transfected control (-) as measured by the ratio between (pTyr-Pro) antibody and total IP SAMHD1 is shown.

ABSENCE of p21 INCREASED HIV-1 REPLICATION AND PROVIRAL DNA FORMATION

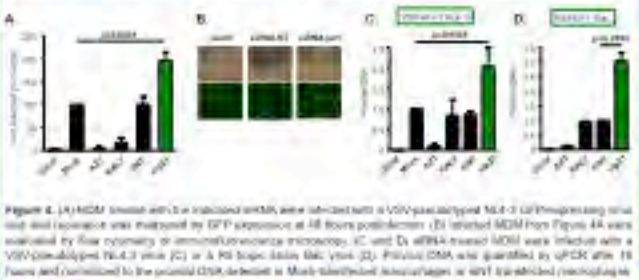


Figure 4. (A) MDM treated with 5 nM indicated siRNA were infected with a VSV-pseudotyped NL4-3 GFP-expressing virus and viral replication was measured by GFP expression at 48 hours post-infection. (B) Infected MDM from Figure 4A were evaluated by flow cytometry or immunofluorescence microscopy. (C and D) siRNA-treated MDM were infected with a VSV-pseudotyped NL4-3 virus (C) or a R5 tropic clone BaL virus (D). Proviral DNA was quantified by qPCR after 16 hours and normalized to the proviral DNA detected in Mock-transfected monocytes or MDM transfected monocytes.

p21 CONTROL of SAMHD1 is DEPENDENT on VPX

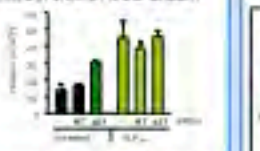


Figure 5. Infection of siRNA-treated MDM in the presence or not of viral-like particles carrying Vpx.

A MODEL for p21 ROLE IN SAMHD1 FUNCTION



Figure 6. p21 is a known inhibitor of CDKs shown to regulate CDK1/2 function in cell culture and in vivo. In vitro it has been shown that SAMHD1 activity is controlled by CDK through phosphorylation leading to SAMHD1 tetramerization and increased dNTP levels required for infection. Conversely RNR2 increases dNTP pool more directly since RNR2 function. We conclude that RNR2 may be controlled by p21 upstream of CDK.

ABSENCE of p21 INDUCED AN INCREASE in Ki67 EXPRESSION and SAMHD1 PHOSPHORYLATION

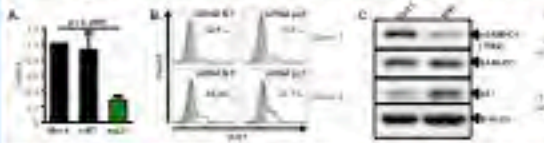


Figure 3. (A) MDM were transfected with a siRNA targeting p21 or a non-targeting siRNA control (siNT). mRNA was quantified by quantitative PCR (qPCR). (B) In the siRNA-treated MDM, Ki67+ cells were evaluated by flow cytometry. (C and D) Lysates of MDM treated with the indicated siRNA were ruined in 12% polyacrylamide gel or 8% polyacrylamide gel and immunoblotted with Ab recognizing the phosphorylated form of SAMHD1 (pSAMHD1) at position T592, anti-SAMHD1, anti-p21, anti-β-actin or Hsp90.

CONCLUSIONS

- p21 affects SAMHD1 restriction of HIV-1.
- This restriction induced by p21 is dependent on CDK2.
- CDK2 controls SAMHD1 function.
- Cell cycle control must include a coordinated regulation of RNR2 and SAMHD1 as both tightly regulate dNTP availability.
- CDK2 activity may be the underlying mechanism explaining p21-mediated control of both RNR2 and SAMHD1 and their control of the dNTP pool required for cell proliferation and virus replication.

See also poster #166 and #168.