

Poster Board Number: 187

Non-integrating HIV-1 is Sensitive to Inhibition by Target Cell APOBEC3A

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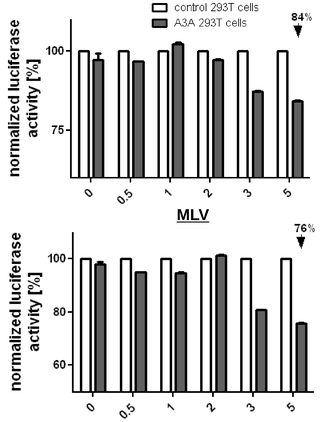
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BACKGROUND

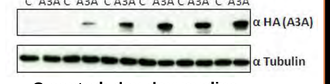
The APOBEC3A (A3A) polynucleotide cytidine deaminase of the human APOBEC3 (A3) protein family was shown to be antiviral active against HTLV-1 but not HIV-1 when expressed in the virus producer cell. In primary monocytes/macrophages, high levels of endogenous A3A activity have been associated with restriction of HIV-1 during infection. So far, ectopic expression of A3A has not been investigated to study the antiviral activity of target cell A3A.

Doxycycline induced target cell A3A inhibits integration deficient HIV-1 and MLV but not wild type HIV-1 effectively

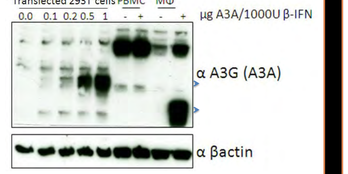
a| HIV-1 integrase mutant D64V



b| A3A level in dox induced 293Tcells



A3A level in transfected cells, PBMC and macrophages

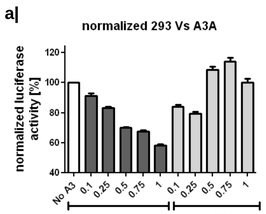


Stable target cell A3A restrict MLV and HIV-1 replication.

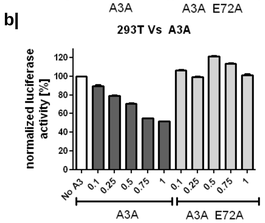
a) Infectivity assay was carried out on cells expressing control/A3A (inducible with doxycycline). Inhibition of MLV and D64V HIV was evident as the luciferase counts got reduced but not wild type HIV. b) A3A expression levels in transfected or stably expressing and primary cells were determined by immunoblots.

APOBEC3A edits plasmid DNA, incorporate efficiently into MLV by interacting with capsid protein, but not into HIV-1 particles

a|



b|

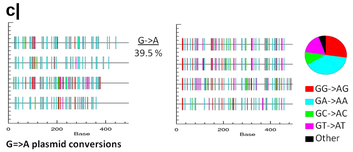


A3A-mediated inhibition of plasmid expression:

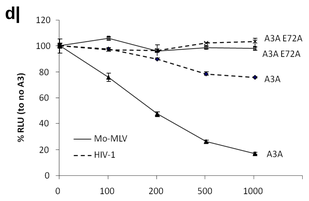
a) HEK293 or b) HEK293T cells (2 x 10^5 cells/well in a 12-well plate) were co-transfected with the luciferase expression plasmid MP71-luc (200 ng), and increasing amounts of the A3A or A3A E72A expression plasmids (0.1, 0.25, 0.5, 0.75 and 1 µg) and pcDNA3.1 empty vector to make up equivalent DNA concentrations for each transfection. Two days post-transfection, luciferase activity was measured to compare the expression of luciferase in different sample.

c) A representation of plasmid editing mediated by A3A in both strands.

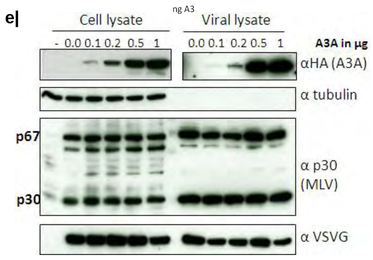
c|



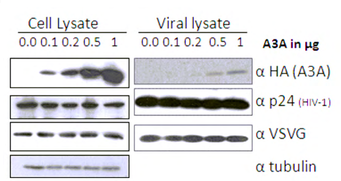
d|



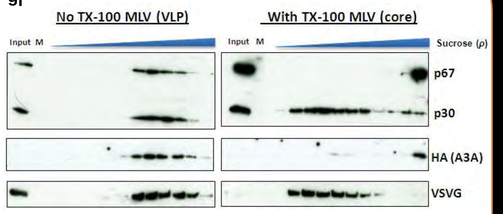
e|



f|



g|

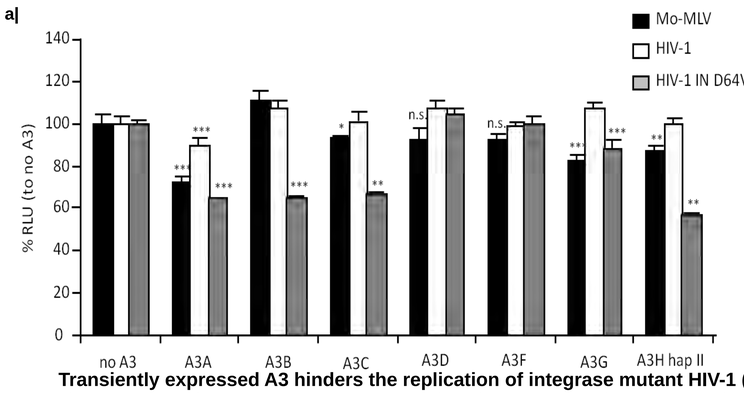


A3A incorporation into MLV core and inhibition activity

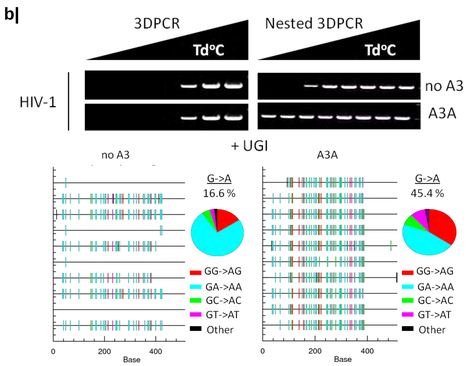
d) Infectivity data suggest that higher the amount of A3A plasmids proportionally reduce the viral replication of MLV but not HIV. Immunoblots of [e)-MLV and f)-HIV] titration experiment with series of A3A plasmid concentration shows the incorporation of A3A into MLV but not in HIV (partially in higher concentration, might be as exosomes) g) sucrose density gradient centrifugation of viral particle compartments show the co-migration of A3A with capsid (core) fraction.

Restriction of HIV-1 integration deficient virus (D64V mutant) by target cell APOBEC3

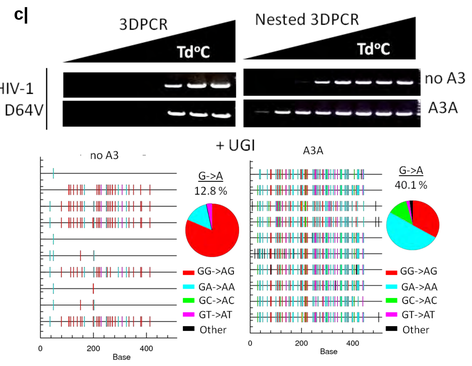
a|



b|



c|



Transiently expressed A3 hinders the replication of integrase mutant HIV-1 (D64V) and MLV

a) MLV, HIV and HIV-1 IN D64V were transduced on cells transiently expressing APOBEC3 proteins. Luciferase assay was carried out five days after transduction and the normalized data presented. b) A3A induced mutations in HIV-1 wt and c) HIV-1 D64V genome. PCR fragments targeted to amplify Luc gene by nested 3D-PCR, amplicons generated at low denaturing temperature were sequenced to determine the A3A triggered G to A conversion, dinucleotide sequence context was presented in the pi chart.

Results and conclusion

We confirm that HIV-1 is insensitive to A3A expressed in the viral producer cells, due to the lack of encapsidation of A3A in viral particles. We hypothesized that HIV-1 may evade the post-entry restriction by target cell A3s by a different timing of integration into the host's genomic DNA. Indeed, when comparing wild type with integrase mutated HIV-1, we observed that the integration-deficient virus was significantly restricted by target cell A3A. Additionally experiments in target cells showed that not only A3A, but also A3B, A3C, A3G, and A3H, but not A3D and A3F, restricted non-integrating HIV-1 post-entry. These data demonstrate that HIV-1's sensitivity to target cell-expressed APOBEC3 proteins is modulated by the integration competence of the virus.

Acknowledgements

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