Analyses of the Sequence-Function Relationships Among HIV-1 Vpr, SIV_{agm}Vpr and SIV_{mac}Vpx

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Background

• Vpr and Vpx proteins share high sequence similarity and both hijack the cellular CRL4 E3 ligase via DCAF1 interaction.

•However, while Vpr induces cell cycle arrest in G2-to-M transition, Vpx degrades cellular SAMHD1 making myeloid cells permissive to viral replication

• Vpx does not induces G2 arrest in infected cells and SIV_{agm}Vpr induces cell cycle arrest in AGM cells but not in human cells

Methods

• Based on the published NMR structure of HIV-1 Vpr, 7 chimeras were generated by exchanging domains between HIV-1 Vpr and SIV_{agm}Vpr and another set of 4 chimeras were generated between HIV-1 Vpr and SIV_{mac}Vpx

• For each chimera we tested the ability to induce cell cycle arrest in human cells and to cause SAMHD1 degradation

•Mapping the domains that are required for the G2 arrest function and SAMHD1 degradation is a great tool analyze the the contribution of such domains to protein function and also for analysis of evolutionary pressures that result in sequence variation

Chimera's schematic representation

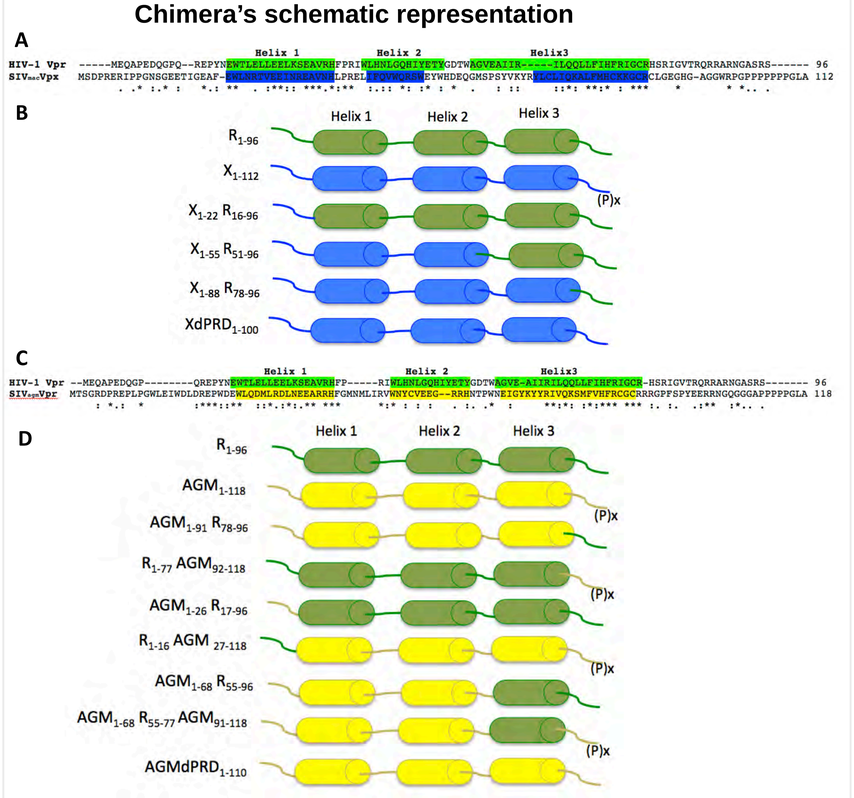


Figure 1: HIV-1 Vpr is comprised of a bundle of three alpha helices flanked by amino-and carboxy-terminal unstructured regions and connected by short flexible loop. The highlighted residues represent the three alpha helices that are symbolized by a barrel shape on the schematic view. Based on the homology between HIV-1 Vpr and SIV_{mac}Vpx and HIV-1 Vpr and SIV_{agm}Vpr, chimeric proteins were constructed **a)** Alignment between HIV-1 Vpr and SIV_{mac}Vpx **b)** Schematic representation of the chimeras between HIV-1 Vpr and SIV_{mac}Vpx **c)** Alignment between HIV-1 Vpr and SIV_{agm}Vpr **d)** Schematic representation of the chimeras between HIV-1 Vpr and SIV_{agm}Vpr.

Transposition of C-terminus of HIV-1 Vpr to SIV_{agm}Vpr confers to SIV_{agm}Vpr the ability arrest human cells in G2-to-M transition

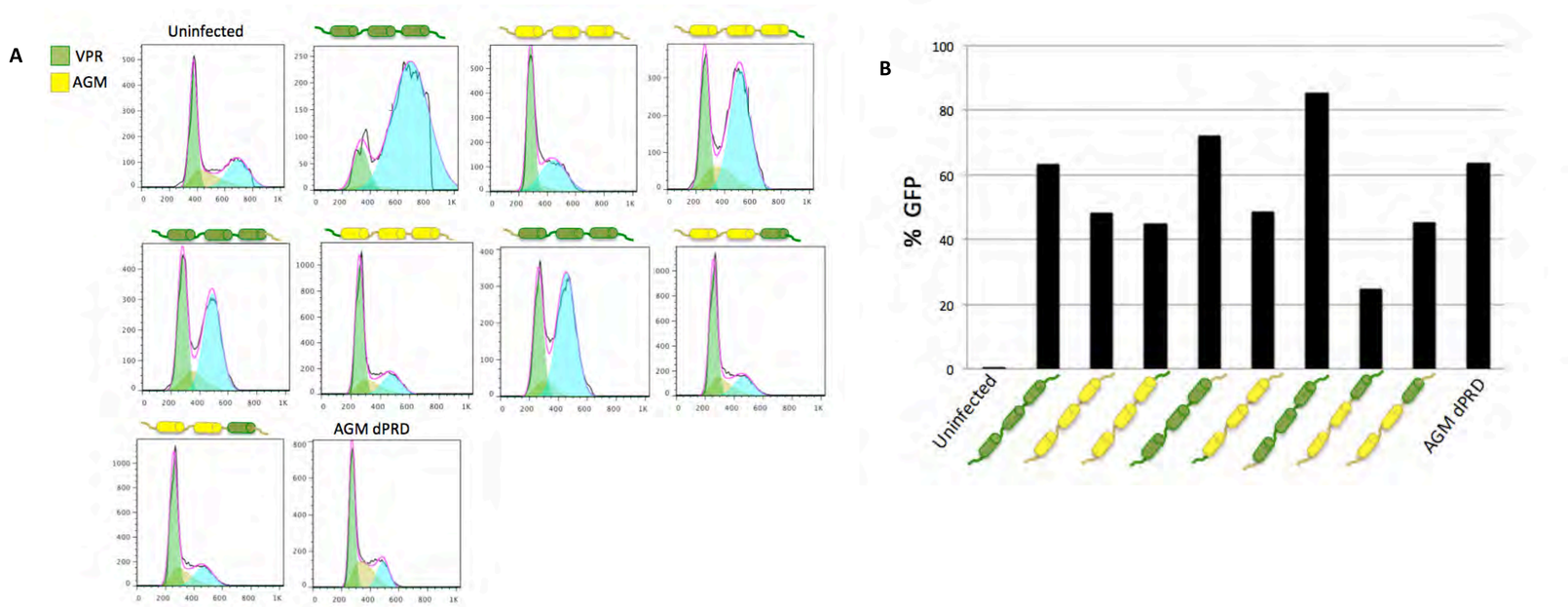


Figure 3: Cell cycle analysis of Hela cells infected with chimeras between HIV-1 Vpr and SIV_{agm}Vpr. **a)** Hela cells were transduced with VSV-G pseudotyped lentiviral for each chimera. 48h p.i DNA staining was used to quantify the cell cycle by flow cytometer **b)** GFP reporter protein that was used to measure the percentage of infection by flow cytometer.

SAMHD1 degradation occurs in a species-specific manner

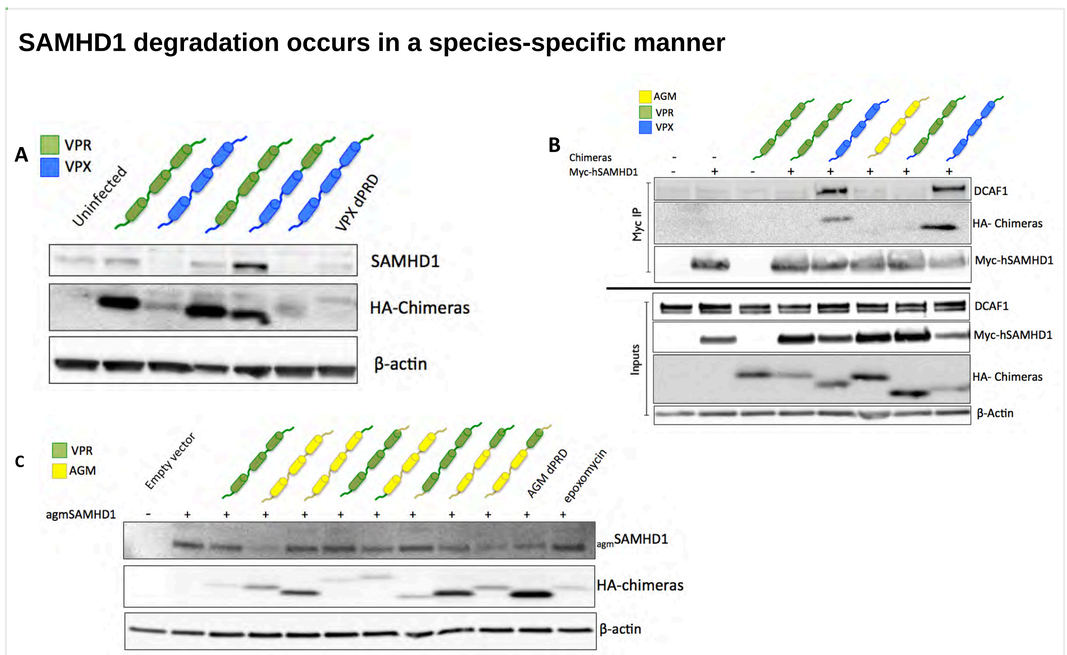


Figure 3: a) Hela cells were infected with the respective chimeras and 48h p.i levels of SAMHD1 were analyzed using anti-SAMHD1 antibody. **b)** 293T cells were transfected with or without myc-hSAMHD1 and in the presence of the respective chimeras. Interactions were addressed by CoIP using anti-myc antibody and magnetic beads. **c)** 293T cells were transfected with myc-$_{agm}$SAMHD1 and the respective chimeras. Levels of agmSAMHD1 were measured by SDS-PAGE.

Conclusions

• Transposition of the C-terminus unstructured region of HIV-1 Vpr to SIV_{agm}Vpr confers to this chimera the ability to induce cell cycle arrest in human cells

• Induction of G2 arrest is not defined by a linear determinant

• The N-terminus unstructured region of SIV_{mac}Vpx is not sufficient for hSAMHD1

•The ability of the chimera to interact with SAMHD1 correlates with SAMHD1 degradation

• The N-terminus unstructured region of SIV_{agm}Vpr is required but no sufficient for $_{agm}$SAMHD1 degradation

References

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