ITIH4: a Cellular Factor Targeted by HIV-1 Vpr

P-A7

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Summary

• The immune system has a multitude of innate immune factors that work to counteract many different viruses. For HIV-1, such factors include APOBEC-3G, Tetherin (BST-2), SAMHD1, and, in the case of non-human primates, Trim5α. However, lentiviruses have evolved accessory proteins to counteract most, if not all of these factors. Such accessory proteins include HIV-1 Vif which degrades APOBEC-3G, HIV-1 Vpu and SIV Nef which reduce cell surface expression of Tetherin, and SIV Vpx which degrades SAMHD1. Interestingly, HIV-1 Vpr, an orthologue of SIV Vpx, does not degrade SAMHD1, and no cellular target for Vpr has been identified as yet.
•Recently, a cell line that is non-permissive to ΔVpr HIV-1 infection has been developed. By examining the proteomic content of viral cores from WT and ΔVpr viruses collected from this cell line, we deduced several potential candidates that could be the culprit to this restriction. One such candidate, the Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) has been investigated in this study. We tested whether Vpr induces degradation of ITIH4. Western Blot analysis of ITIH4 expression was performed on cell lines/primary cells treated or not with IFNα. We also analyzed the effect of ITIH4 overexpression and downregulation on virion production (protein expression and virion release) and infectivity (indicator TZM-bl cells).

•Vpr overexpression resulted in downregulation of ITIH4. Interestingly, ITIH4 overexpression efficiently degraded Vpr, and reduced its incorporation into the virions. Upon siRNA knockdown of ITIH4, Vpr expression and incorporation were increased. When viruses were produced in the presence of ITIH4, a clear inhibition of viral replication was observed at two different points of the viral life cycle. First, there was a block at the production step, with viral production being reduced 3-10 fold. Second, there was a block at the early steps of infection after entry, with a 2-3 fold decrease in infectivity. Western Blot analysis of several cell lines and primary cells demonstrated a lack of this factor within T cells, but showed its presence within THP-1 cells and monocyte-derived macrophages, with upregulation in the presence of IFN-alpha.
Conclusions: The presence of ITIH4 within the producer cell greatly reduces both the production and infectivity of progeny virions. Our data thus warrants further investigation into the exact mechanism by which ITIH4 inhibits HIV and how it interacts with Vpr.

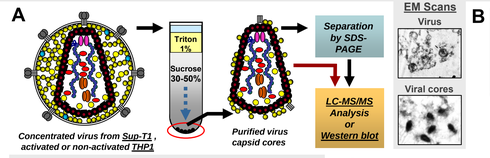
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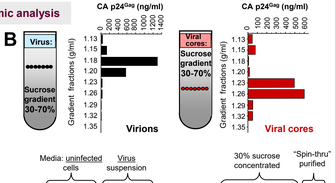
Background

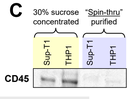
HIV-1 viral particles released from infected cells incorporate many cellular proteins during the initial steps of morphogenesis – assembly and budding. Findings from earlier studies identified more than 300 cellular proteins in HIV-1 particles (summarized at http://web.ncifcrf.gov/research/avp/protein_db.asp). HIV-1 hijacks components of the cellular *endosomal sorting machinery* and *cytoskeleton*, *surface proteins*, *RNA-binding proteins* associated with incorporated RNA molecules, *chaperones* and multiple concomitant proteins. Upon fusion of an HIV-1 particle with a target cell, viral cores are released into the cytoplasm and rearranged into sub-viral particles – RTCs. These structures support reverse transcription and protect and transport viral cDNA to the site of integration. RTCs are composed of both viral and cellular proteins. Since RTCs are formed from the viral cores, their initial composition is identical to that of viral cores. Although the key early steps of HIV replication, reverse transcription and integration, are relatively autonomic, participation of cellular proteins in these events has been demonstrated. However, despite the fact that numerous cellular proteins have been identified in HIV-1 particles, the functions of the most of them in the early infection stages remain unknown.

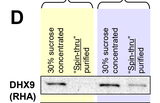
Technical Approach

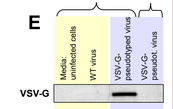
1. "Spin-thru" purification allows isolation of cores from HIV-1 virions for the proteomic analysis











A. Scheme of the isolation of central cores from HIV-1 virions by "spin-thru" detergent-layered density gradient centrifugation of the concentrated virions.

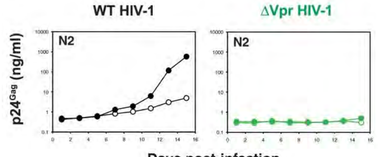
B. "Spin-thru" purification effectively purifies viral cores (density 1.23-1.27 g/ml) from whole virions (density 1.14-1.2 g/ml) – p24 ELISA of the fractions of sucrose density gradients after centrifugation of concentrated HIV-1 NL4-3 virions and cores.

C, D. "Spin-thru" separates viral cores from extracellular vesicles – Western blot detection of CD45 (**C**) and DEAD box RNA helicase A (DHX9) (**D**) in core preparations from the virus produced by infected Sup-T1 and THP1 cells.

E. "Spin-thru" separates cores from the viral envelope – Western blot detection of VSV-G glycoprotein in the samples of VSV-G-pseudotyped HIV-1 NL4-3 before and after "spin-thru" centrifugation

Results

2. LC-MS/MS analysis revealed several cellular proteins that were present in the cores of ΔVpr HIV-1 virions produced by the non-permissive N2 cell line, but absent in the cores of WT virions produced from the same cell line.



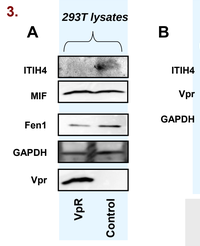
Accession	# AAs	MW [kDa]	Description	Score ΔVpr	Coverage	# Peptides	Score WT	Coverage	# Peptides
gi4506355	1482	163.7	pregnancy-zone protein	22.90	2.97	4			
gi31542984	**930**	**103.3**	**inter-alpha (globulin) inhibitor H4**	**14.19**	**1.94**	**2**			
gi125604	531	57.9	Pyruvate kinase, M2 isozyme	5.58	5.84	3			
gi24308125	962	106.8	KIAA1280 protein	5.56	1.46	1			
gi2521981	334	35.6	alpha2-HS glycoprotein	5.54	7.49	3			
gi20555668	216	24.3	similar to Chain A, Canine Gdp-Ran Q69l Mutant	5.22	11.57	2			
gi1620909	1006	115.4	ceruloplasmin	5.01	0.99	1			
gi4505763	417	44.6	phosphoglycerate kinase 1	4.40	5.28	2			
gi4505185	**115**	**12.5**	**macrophage migration inhibitory factor**	**4.39**	**7.83**	**1**			
gi30149784	395	42.6	similar to elongation factor 1 alpha	3.74	4.81	2			
gi2119925	227	24.2	translation elongation factor eEF-1 alpha - human (fragment)	3.74	7.93	2			
gi28527521	**380**	**42.6**	**similar to flap structure-specific endonuclease 1**	**3.22**	**1.84**	**1**			
gi10092579	**885**	**99.1**	**pre-alpha (globulin) inhibitor, H3 polypeptide**	**2.63**	**2.26**	**2**			
gi31543397	417	44.8	phosphoglycerate kinase 2	2.40	4.80	2			
gi24234686	493	53.5	heat shock 70kDa protein 8 isoform 2	2.30	4.46	2			
gi29734845	385	41.3	similar to Heat shock protein HSP-2 (hsp-2) [Homo sapiens]	2.30	6.23	2			

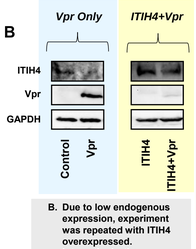
ITIH4: **inter-alpha-trypsin inhibitor 4:**
•Known to be primarly expressed in the liver
•Secreted into the blood, and identified as an anti-inflammatory and acute-phase protein.
•Infection of cattle with BRSV has been shown to result in high ITIH4 levels

MIF: **Macrophage migration inhibitory factor:**
•Secreted by immune cells upon the exposure to foreign antigens
•Known to be an important regulator of innate immunity
•Identified as an pro-inflammatory cytokine

FEN1: **Flap endonuclease 1:**
•Known to process DNA fragments during DNA synthesis
•Already hypothesized to have a role in HIV infection during nuclear import/integration

3.

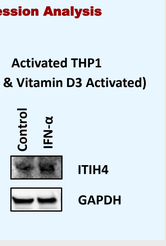


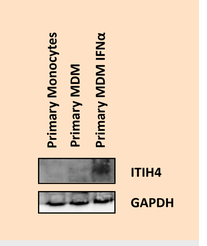


A. Western blot analysis of selected cellular proteins in 293T cells transfected with Vpr.

B. Due to low endogenous expression, experiment was repeated with ITIH4 overexpressed. Interestingly, the overexpression of ITIH4 significantly reduced Vpr expression

4. Expression Analysis

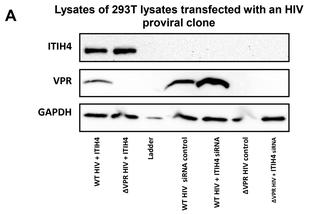


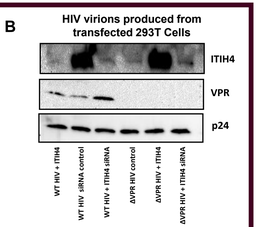


While undetectable in T cells, ITIH4 is clearly upregulated in Monocyte-derived Macrophages in the presence of IFN-α

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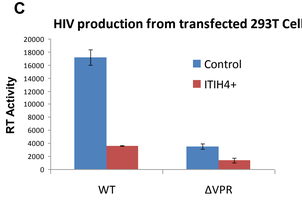
A. ITIH4 protein levels were manipulated in virus producing 293T cells by plasmid transfection (overexpression) or siRNA transfection (knockdown). ITIH4 levels were then confirmed, and Vpr levels were also assessed.
B. Virions produced from the aforementioned cells were also assessed for their ITIH4 and Vpr levels.

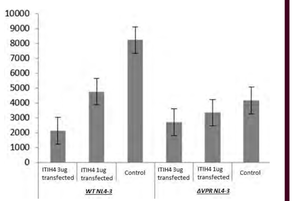




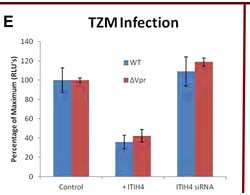
Both viral production (C) and infectivity of progeny virions (D) produced in the presence of overexpressed ITIH4 was significantly reduced.

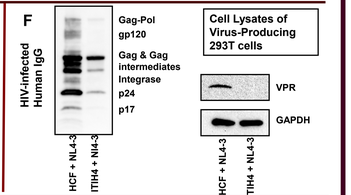
- **Of note, no differences were seen in the presence of ITIH4 siRNA, most probably due to the low endogenous expression within 293T cells**





E. The requirement of ITIH4 to be present within cores to exert its post-entry block was assessed by overexpressing ITIH4 within TZM cells and infecting them.
F. HIV protein expression was assessed by immunoblot when ITIH4 was overexpressed, demonstrating a broad decrease in overall HIV protein production.





Discussion

- **Analysis of ΔVpr viral cores produced by a non-permissive NKR cell clone revealed a cellular factor, ITIH4, that is targeted by Vpr and possibly responsible for the infection block seen within this cell line. At sufficient concentrations it appears that Vpr expression is reduced in the presence of ITIH4 as well. This factor is also present within relevant HIV target cells, specifically macrophages, and is up-regulated by IFN-α.**
•The infection block exerted by ITIH4 occurs at two steps of the HIV life cycle. The production step is inhibited by ITIH4 3-10 fold, apparently due to a broad decrease in HIV protein production. The second block occurs post-entry; interestingly, it appears that ITIH4 does not need to be within the viral core to restrict infection (unlike APOBEC3G), and if ITIH4 concentration is sufficiently high within target cells, infection is restricted.
•Further studies are warranted to elucidate the exact mechanisms of ITIH4 effects on production and post-entry steps of HIV replication.

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