Queen Mary University of London

Blizard Institute

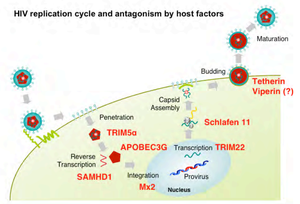
Barts and The London School of Medicine and Dentistry

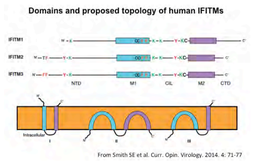
Interferon-Induced Transmembrane Proteins (IFITMs) Antagonize Postintegration Replication of HIV but Are Overcome by Viral Membrane Accessory Proteins

Wing-Yiu Jason Lee, Ifrah Omar and Richard Sloan

Introduction

- IFITMs are up-regulated in macrophages and T cells by interferons.
- Genome scale RNA interference screens and screens of interferon-stimulated genes identified IFITMs as restriction factors of virus entry.
- However, it has become apparent that IFITMs affect a late stage of HIV replication also, distinct from their defined effect on entry.
- We aim to study the effect of IFITMs on the post-entry steps of HIV replication cycle.





Expression of IFITMs reduces the level of HIV and SIV particles released from transfected cells and infected T cells

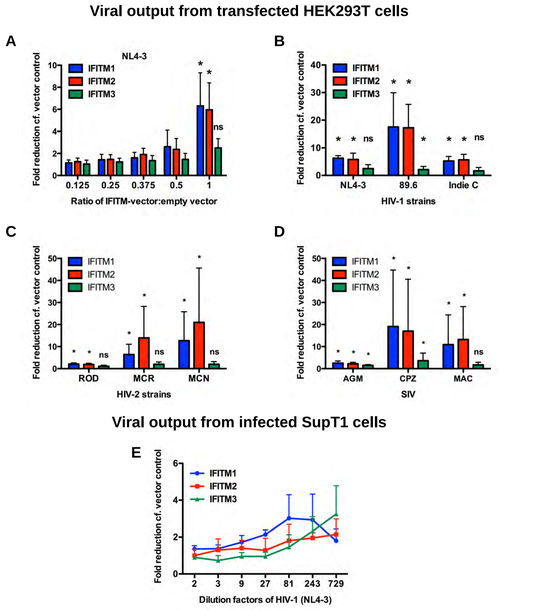


Figure 1A. HEK 293T cells were transfected with proviral DNA encoding for the genome of **(A, B)** HIV-1, **(C)** HIV-2, **(D)** SIV and a mixture of human IFITM-encoding vector and empty vector. **(E)** SupT1 cell lines expressing individual IFITMs were infected with wildtype NL4-3 virus at indicated dilutions. 48 hours post-transfection/infection, the quantity of virus particles released into the supernatant was measured by p24 ELISA in **A**, **B** and **E** and reverse transcriptase activity assay in **C** and **D**. The fold reduction of virus particle output from IFITM-expressing cells was compared to vector control using non-parametric paired t tests. Data show mean+S.D. from 3 experiments. * denotes p<0.05 and ns denotes p>0.05 in non-parametric unpaired t tests.

IFITMs interfere with translation of viral transcripts and reduce the expression of viral proteins

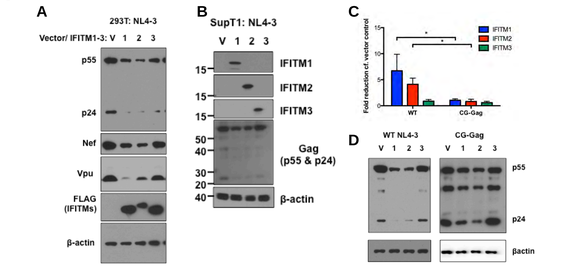
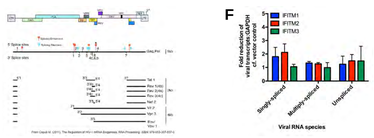
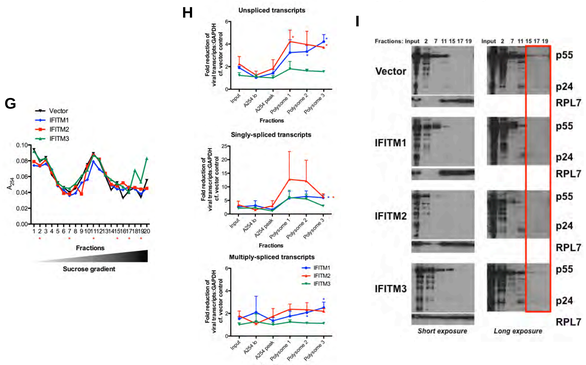


Figure 2. IFITMs suppress viral protein expression and codon-optimization of Gag relieves IFITM inhibition. (A) HEK293T cells transfected with proviral wildtype NL4-3 and with either human IFITM-encoding vectors or empty vector. **(B)** IFITM-expressing SupT1 cells were infected with NL4-3 virus for 48 hours. Cell lysates were analyzed by immunoblotting with antibodies specific for Gag (p55 as precursor and p24 as mature capsid protein), Nef, Vpu and IFITMs (FLAG-tagged in **A**. 293T cells or monoclonal antibodies were used in **B**). **(C)** Viral output from HEK293T cells transfected with either NL4-3 proviral DNA or pCNCGP1, a proviral DNA of NL4-3 with codon-optimized Gag (CG Gag) and plasmids encoding IFITM1/2/3 was measured by p24 ELISA and Gag expression was examined by immunoblotting in **(D)**. * denotes p<0.05 in non-parametric unpaired t tests.



IFITMs do not have a major effect on viral transcript expression. (E) Schematic diagram of HIV-1 RNA transcripts. **(F)** Fold change of normalized viral transcript levels in HKE293T cells 48 hours post-transfection with NL4-3 proviral DNA and either vector or IFITM expression plasmids, measured by quantitative PCR.

Levels of viral transcripts and proteins in polysome analysis



IFITMs repress translation of viral transcripts. (G) Absorbance (254nm) profile of cell lysate fractions from HEK293T cells transfected with NL4-3 proviral DNA and vector/IFITM expression plasmids, analyzed by sucrose gradient ultracentrifugation. Indicated fractions (*) were further analyzed for levels of viral transcripts by quantitative PCR**(H)**. Data show mean+S.D. of fold reduction of GAPDH-normalized viral transcripts in IFITM co-expressing cells versus vector control from 3 experiments. Colored asterisks indicate p<0.05 in comparison to input fractions by non-parametric unpaired t test. **(I)** Level of Gag in polysome fractions was analyzed by immunoblotting and ribosomal protein (RPL7) was used as loading control.

IFITMs associate with viral transcripts and DEAD-box RNA helicase, DDX6

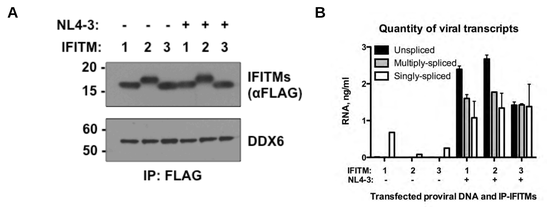


Figure 3. (A) FLAG-tagged IFITM were immunoprecipitated from HEK293T cells transfected with human IFITM-encoding vectors and/or proviral DNA encoding for the genome of wildtype NL4-3. Levels of immunoprecipitated IFITMs and co-immunoprecipitated DDX6 were analyzed by immunoblotting. Data show representative results from 3 experiments. **(B)** RNA isolated from immunoprecipitated IFITMs was analyzed for viral transcripts by quantitative PCR. Data shown are mean+S.D. from one experiment.

Viral proteins, Nef and Vpu, antagonize antiviral activity of IFITMs

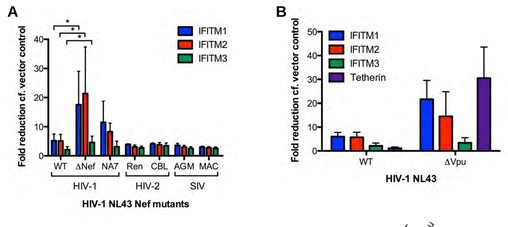


Figure 4. Nef and Vpu antagonize antiviral activity of IFITMs and this antagonism is ubiquitous in lentiviral Nef proteins. HEK293T cells were transfected with wildtype/ Nef-mutants/ Vpu-deleted NL4-3 proviral DNA and empty vector or human IFITM-encoding vectors. **(A and B)** The quantity of virus released 48 hours post-transfection was measured by p24 ELISA and normalized to empty vector transfected control. Data shown mean+.S.D. from 3 experiments. **(C)** Cell lysates were analyzed for expression of Gag and IFITMs (FLAG) by immunoblotting. Tetherin was used as a positive control and β-actin was used as a loading control.

Palmitoylation-deficient mutants of IFITMs show enhanced antiviral activity

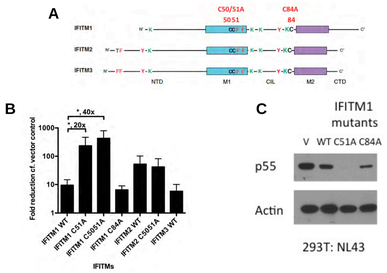
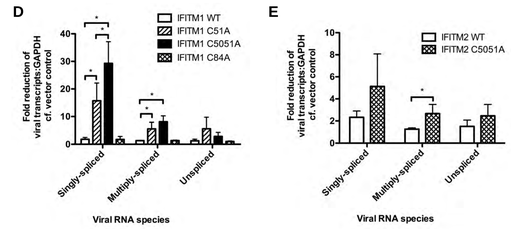
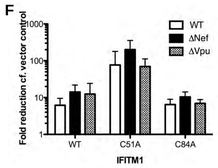


Figure 5. HEK293T cells were transfected with wildtype NL4-3 proviral DNA and empty vector or human IFITM-encoding vectors with indicated mutations (**A**, in red). **(B)** The quantity of p24 of released virus was measured by ELISA 48 hours post-transfection and the fold reduction normalized to vector control as mean+S.D. is shown. * denotes p<0.05 in non-parametric unpaired t tests. **(C)** Cell lysates were analyzed for Gag expression by immunoblotting.



Palmitoylation-deficient mutants of IFITM1 and 2 reduce the level of viral transcripts
HEK293T cells were transfected with wildtype NL4-3 proviral DNA and empty vector or human IFITM-encoding vectors with indicated mutations in **(A)** IFITM1 and **(B)** IFITM2. RNA was isolated 48 hours post-transfection and was analyzed for the levels of viral RNA using quantitative PCR. The level of viral RNA was first normalized with the level of GAPDH RNA in the corresponding sample and then compared to vector control. *denotes p<0.05 in non-parametric unpaired t tests.



C51A palmitoylation-deficient mutant of IFITM1 overcome antagonism by Vpu but not Nef. HEK293T cells were transfected with wildtype/Nef-deleted/Vpu-deleted NL4-3 proviral DNA and empty vector or human IFITM-encoding vectors with indicated mutations in IFITM1. Viral output in supernatant was analyzed by p24 ELISA 48 hours post-transfection. Data show fold reduction of p24 as mean+S.D. compared to vector control.

Conclusions

- We describe a novel role of IFITMs in restricting HIV-1 replication by suppression of viral translation. This is in addition to their role in restricting viral entry.
- Majority of HIV-1, HIV-2 and SIV strains tested are susceptible to human IFITMs, though not all (HIV-2 ROD10 and SIV AGM-tan)
- Association of IFITMs with viral RNAs and DDX6 suggests that IFITMs may suppress viral translation via host helicases.
- Lentiviral accessory proteins Nef and Vpu can both have the capacity to act as IFITM countermeasures.
- All lentiviral Nef alleles tested have the capacity to act as IFITM countermeasures.
- The varying ability of Nef and Vpu to overcome the highly potent IFITM1 mutant C51A suggest differing capacity to counter IFITMs.
- Highly active non-palmytoylated IFITM mutants may be explored therapeutically, or to further dissect IFITM biology.