Antagonism of Viral Glycoproteins by Guanylate Binding Protein 5

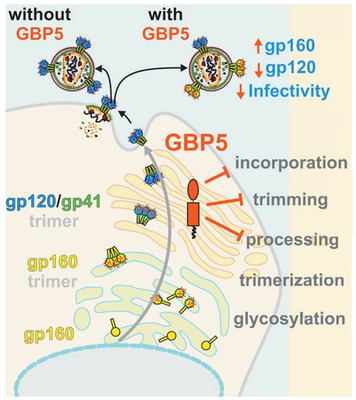
Hana Veler[1], Geraldine V. Vilmen[1], Abdul A. Waheed[1], Chen Man Lun[1], and Eric O. Freed[1]

[1]Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD, USA

306 CROI Conference on Retroviruses and Opportunistic Infections

Introduction

- Guanylate binding protein (GBP) 5 is an interferon-inducible cellular factor with broad antiviral activity, reducing the infectivity of progeny virions by interfering with processing and incorporation of viral glycoproteins.
- GBP5 is believed to inhibit the infectivity of viruses such as HIV-1[1] and influenza A virus[2] by reducing the proteolytic activity of furin.

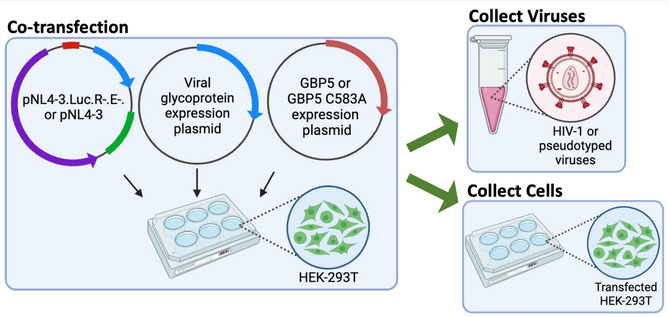


GBP5 reduces HIV-1 infectivity by reducing the proteolytic activity of furin.[1]

Objectives

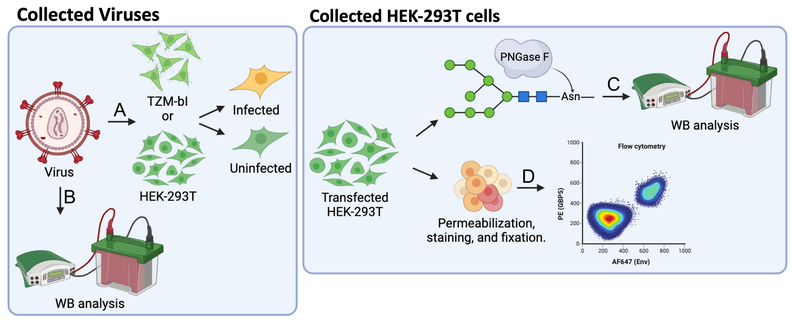
- Determine mechanism by which GBP5 inhibits processing of viral glycoproteins.
- Determine whether GBP5 affects only furin-dependent glycoproteins.

Methods



pNL4-3 was co-transfected with either GBP5 or the inactive GBP5 C583A mutant expression plasmid in 293T cells; HIV-1 Δenv luciferase reporter viruses were pseudotyped with either SARS-CoV spike (S) or SARS-CoV-2 S.[3]

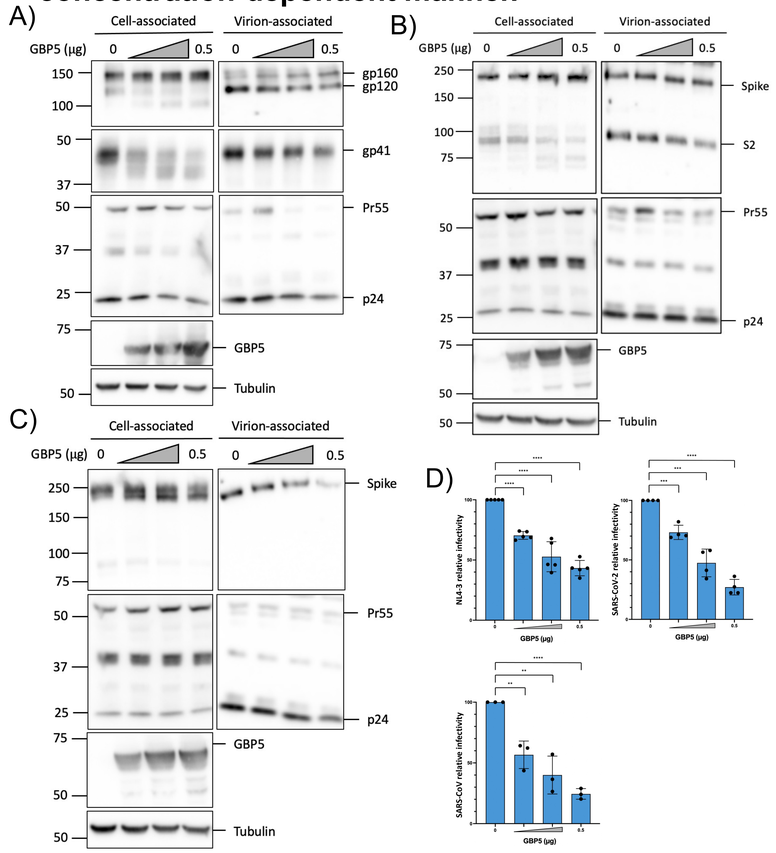
Methods



(A) The effect of 293T producer-cell GBP5 expression on particle infectivity was determined. Virus- (B) and cell-associated (C) proteins were analyzed by Western blot. (D) HIV-1 Env cell surface expression was measured by flow cytometry.[3]

Results

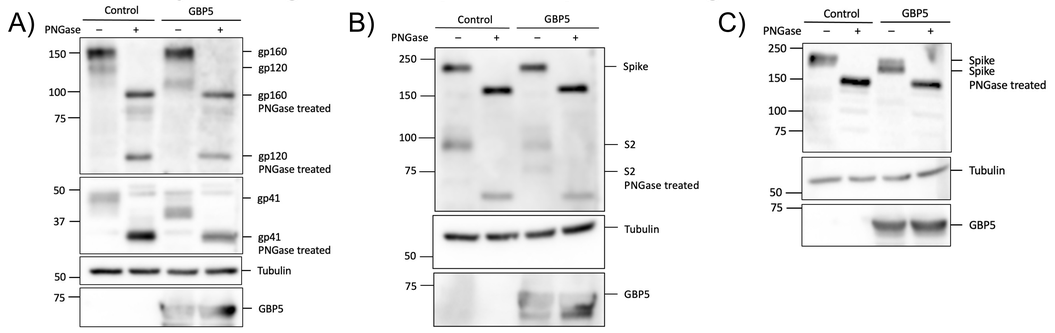
1. GBP5 reduces HIV-1 Env, SARS-CoV S, and SARS-CoV-2 S incorporation and virion infectivity in a concentration-dependent manner.



Virus- and cell-associated proteins of HIV-1 (A) and SARS-CoV-2 S (B) or SARS-CoV S (C) pseudotyped viruses were analyzed by Western blot. (D) Particle infectivity was was determined. Data are representative of either three or four independent experiments.

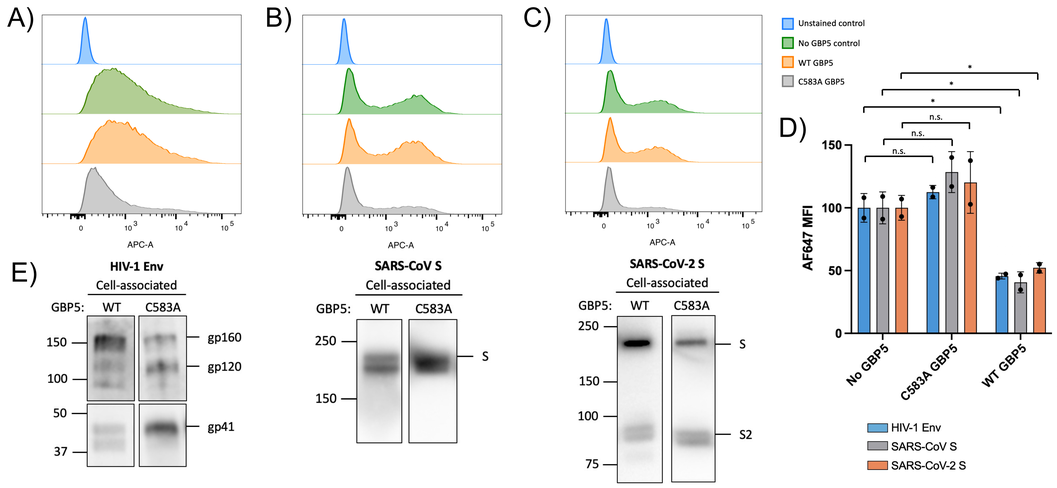
Results

2. GBP5 reduces glycosylation of viral glycoproteins independent of whether they undergo furin-dependent processing.



The effect of GBP5 expression on HIV-1 Env (A), SARS-CoV-2 S (B), and SARS-CoV S (C) viral glycoprotein glycosylation was determined by treating virus-producing cell lysates with N-glycosidase F (PNGase F), which removes N-linked oligosaccharides from glycoproteins.

3. GBP5 reduces cell surface expression of HIV-1 Env, SARS-CoV S, and SARS-CoV-2 S.



The expression of cell surface HIV-1 Env (A), SARS-CoV S (B), and SARS-CoV-2 S (C) in the presence of either GBP5 or inactive C583A GBP5 mutant was determined by flow cytometry. APC: glycoprotein. Data are representative of two independent experiments. (D) AF647 (glycoprotein) median fluorescence intensity (MFI) was then calculated for each GBP5 protein. (E) Cell-associated viral glycoprotein proteins were analyzed by Western blot.

Conclusions & Future Directions

- GBP5 reduces HIV-1 Env, SARS-CoV-2 S, and SARS-CoV S incorporation and cell surface expression, and virion infectivity in a concentration-dependent manner.
- GBP5 not only inhibits furin cleavage of viral glycoproteins but also disrupts their glycosylation independent of whether they undergo furin processing.
- Future work will elucidate mechanisms of viral evasion from GBP5 restriction, investigate the effect of GBP5 expression on viral glycoprotein trafficking by immuno-fluorescence microscopy, and study the role of GBP5 in viral pathogenesis *in vivo*.

[1] Krapp *et al.*, Cell Host Microbe, 2016.
[2] Feng *et* al., J Innate Immun, 2017.
[3] Figures created with BioRender.com

NIH NATIONAL CANCER INSTITUTE