Biochemical and Biophysical Research Communications 448 (2014) 95–100

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Interferon-mediated ISG15 conjugation restricts dengue virus 2 replication

Takayuki Hishiki [a,b], Qi'En Han [a], Kei-ichiro Arimoto [c], Kunitada Shimotohno [d], Tatsuhiko Igarashi [b], Subhash G. Vasudevan [e], Youichi Suzuki [a,e,*], Naoki Yamamoto [a,*]

[a] Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, 14 Medical Drive,Singapore 117599, Singapore
[b] Laboratory of Primate Model, Experimental Research Center for Infectious Diseases, Institute for Virus Research, Kyoto University, Kyoto 606-8517, Japan
[c] Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA
[d] Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Chiba 272-8516, Japan
[e] Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, 8 College Road, Singapore 169857, Singapore

ARTICLE INFO

Article history:
Received 28 March 2014
Available online 21 April 2014

Keywords:
Dengue virus
ISG15
ISGylation
Interferon
Non-structural protein
Antiviral response

ABSTRACT

ISGylation, an ubiquitin-like post-translational modification by ISG15, has been reported to participate in the interferon (IFN)-mediated antiviral response. In this study, we analyzed the functional role of ISGylation in dengue virus 2 (DENV-2) replication. Overexpression of ISG15 was found to significantly suppress the amount of extracellular infectious virus released, while intracellular viral RNA was unaffected. This effect was not observed with a conjugation-defective ISG15 mutant. In addition, extracellular virus infectivity was decreased by ISG15 overexpression. To further clarify the role of ISGylation in the anti-DENV-2 response, we depleted endogenous ISG15 by RNA interference and analyzed the virus production in the absence or presence of type-I IFN. Results showed a significant reduction in extracellular DENV-2 RNA levels for cells treated with IFN, and that these DENV-2 RNA levels could be partially restored by the ISG15 knockdown. Among various DENV-2 proteins, NS3 and NS5 were subjected to the ISGylation. These results demonstrate that IFN-inducible ISGylation suppresses DENV-2 particle release, and that ISG15 is one of the mediators of IFN-induced inhibition of DENV-2 replication. ISG15 therefore functions as a host antiviral factor against DENV-2 infection.

1. Introduction

Dengue is the most common arthropod-borne viral infection in the world. Dengue virus (DENV) is found in more than 100 countries and is still spreading to new areas every year [1]. The presence of four antigenically distinct serotypes of DENV (DENV-1 to 4) is important with regard to the clinical manifestations of dengue. Primary infection with one serotype is often asymptomatic or manifests as self-limiting dengue fever (DF), but antibodies produced during this primary infection are non- or sub-neutralizing to heterologous serotypes and can enhance secondary infection, resulting in more severe and life-threatening manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2].

DENV is an enveloped, single sense positive-strand RNA virus belonging to the *Flaviviridae* family. The viral RNA is translated at the endoplasmic reticulum to produce a polyprotein, which is further processed by cellular and viral proteases into three structural proteins (C, prM and E) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The structural proteins are assembled into the virus particle, while the NS proteins are responsible for the replication of the viral RNA genome [3].

The primary targets of virus infection in humans are the monocyte lineage cells, including dendritic cells and macrophages [4]. Although these cells play an important role in the innate and protective immunity against virus infections, the mechanisms of immunopathogenesis and the host immune response in DENV infection remain largely unknown. Since no approved vaccines or antiviral drugs are available for the prevention or treatment of dengue, understanding the immune response during DENV infections will contribute to the development of DENV therapeutics.

Induction of the interferon (IFN) response is central to host protective immunity and its signaling pathways have an essential function in the triggering of innate immune responses against pathogens [5]. DENV infection is a weak inducer of the IFN

* Corresponding authors at: Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, 14 Medical Drive, Singapore 117599, Singapore. Fax: +65 6776 6872.
E-mail addresses: yoichi.suzuki@duke-nus.edu.sg (Y. Suzuki), micny@nus.edu.sg (N. Yamamoto).

http://dx.doi.org/10.1016/j.bbrc.2014.04.081
0006-291X/

responses and can block IFN signaling pathways to some extent [6], but treatment with type-I and type-II IFNs can limit virus replication *in vitro* during the initial stage of DENV infection [7]. In addition, it has been reported that DF patients have higher levels of circulating IFN-α and IFN-γ as compared to DHF/DSS patients, suggesting that IFN levels are one of the determinants of clinical outcome in DENV infection [8]. Furthermore, DENV is only able to replicate in mice deficient in IFN receptors [9] or an IFN signaling component, STAT2 [10]. These studies indicate that the IFN system is part of the host protective immune response that controls DENV replication and pathogenesis.

It is well known that the anti-viral effects of IFN are mediated by IFN-stimulated genes (ISGs), some of which are also induced by virus infection [11]. Several ISGs have also been reported to inhibit distinct steps of the DENV replication cycle, though the suppression mechanisms remain unclear [12,13]. ISG15 is a type-I IFN inducible, 17 kDa protein that contains two ubiquitin-like domains. Like ubiquitin, ISG15 exists as a free molecule and can be conjugated to other proteins. In human cells, the ISG15 conjugation (ISGylation) is catalyzed by Ube1L (E1 enzyme), UbcH8 (E2 enzyme), and EFP/HERC5 (E3 enzymes), which are also induced by type-I IFN. Importantly, ISGylation has been demonstrated to cause either a gain or loss of function in target proteins [14].

Negative regulation of viral replication by ISGylation has been reported in human immunodeficiency virus, influenza virus and hepatitis C virus [15–17]. With respect to flaviviruses, a recent study using a mouse macrophage cell line showed that the depletion of ISG15 by small interference RNA (siRNA) increased DENV and West Nile virus (WNV) replication [18]. However, the detailed mechanism underlying the anti-DENV effect of ISG15 remains unclear. In the present study, we report that ISG15 is one of the mediators of type-I IFN-induced inhibition of DENV replication in human cells, and that ISGylation was indispensable for the ISG15-mediated anti-DENV effect, which was likely to suppress viral particle release.

2. Materials and methods

2.1. Cells and viruses

Human cervical cancer cells (HeLa), and human embryonic kidney cells (HEK293T) were grown in Dulbecco's modified Eagle's medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 100 μg/ml penicillin and 100 μg/ml streptomycin sulfate. Baby hamster kidney cells (BHK-21) were grown in Roswell Park Memorial Institute 1640 medium (RPMI, Life Technologies) supplemented with 10% FBS and antibiotics. All cells were incubated at 37 °C with 5% CO_2. *Aedes albopictus* mosquito cells (C6/36) were maintained at 28 °C in HEPES-modified RPMI supplemented with 10% FBS and antibiotics.

High-titer stock of DENV-2 was prepared by inoculating C6/36 cells with DENV-2, which had been isolated in Singapore [19], at a multiplicity of infection (MOI) of 0.1 and cultured for 5 days. The supernatants were collected and centrifuged at 1000 × *g* for 5 min. Infectious viral titer was determined by plaque assays using BHK-21 cells as described below.

2.2. Plaque assay

BHK-21 cells were seeded in 24-well plates at 1.5×10^5 cells/well. Serial dilutions of virus supernatants were added and incubated at 37 °C for 1 h, followed by addition of RPMI containing 2% FBS, antibiotics and 0.8% carboxymethylcellulose (CalBiochem) to each well. After 5 days of incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde at room temperature for 20 min. The plaques were visualized by staining with 1% crystal violet. Virus titer was calculated as plaque-forming units (PFU) /ml.

2.3. Plasmid DNA

cDNAs of DENV structural and NS proteins were amplified either from pXJ-C-prM-E (encoding structural genes [20]) or pWSK-DENV-Rep (encoding NS genes [20]) by PCR and cloned into the *Not*I-*Xho*I site of pCAG vector containing N- (for C, NS2B and NS3 expressions) or C-terminal FLAG (for prM, NS1, NS4A, NS4B and NS5 expressions) sequence. The expression plasmids of human ISG15 (ISG15-pCAG-HA), Ube1L (Ube1L-pcDNA3) and Ubch8 (Ubch8-pcDNA3) have been described previously [21]. Site-directed mutagenesis of the ISG15 gene was carried out by inverse PCR method [22] (ISG15-AA-pCAG-HA).

2.4. Intracellular and extracellular DENV infectivity assay

The DENV infectivity assay was performed as described previously [23] with slight modifications. HeLa cells (4×10^5 cells in a 60 mm dish at 1 day prior transfection) were transfected with 5 μg of ISG15-pCAG-HA, 1.5 μg of Ube1L-pcDNA3 and 1.5 μg of Ubch8-pcDNA3. Four hours after transfection, cells were inoculated with DENV-2 at MOI of 10 for 2 h. Inoculated cells were washed three times with PBS, and then cultured for 36 h. Infected cells were washed twice with PBS, collected by centrifugation, and suspended in distilled water. Cells were then sheared by 10 strokes with a 22-gauge needle before incubation at room temperature for 15 min. The lysate supernatant was collected after centrifugation at 15,000 × *g* for 5 min, filtered through a 0.45-μm filter (Millipore), and concentrated with a Vivaspin-6 centrifugal filter (GE Healthcare). The solvent was exchanged with DMEM, and the resulting solution was used for the intracellular DENV quantification. Culture supernatant at 36 h after infection was used to quantify the extracellular virus titer. Virus titer in each sample was analyzed by plaque assay as described above.

2.5. RNA interference experiment

A siRNA duplex targeting human ISG15 (si-ISG15: 5′-UGAGCAC CGUGUUCAUGAA-3′) and a negative control siRNA duplex (si-control) were purchased from Integrated DNA Technologies. HeLa cells (2×10^4 cells/well at 1 day prior transfection), were transfected with 50 nM si-ISG15 or si-control using Silentfect (Bio-Rad). Four hours after transfection, cells were treated with 1000 units/ml type-I IFN (a mixture of human interferon α and ω, Sigma) for 8 h, and then inoculated with DENV-2 at a MOI of 1. Intracellular and extracellular DENV RNA were extracted 36 h after infection and quantified by real-time RT-PCR analysis (see below). Expression levels of ISG15 were analyzed by immunoblotting at 24 h after IFN treatment.

2.6. Immunoblotting analysis

Protein samples were separated by 7% SDS–PAGE gel and transferred to Immobilion P transfer membrane (Millipore). The primary antibodies used were anti-HA rabbit monoclonal (C29F4, Cell Signaling), anti-FLAG mouse monoclonal (M2, Sigma), anti-actin mouse monoclonal (AC40, Sigma), and anti-ISG15 rabbit polyclonal (2743, Cell Signaling) antibodies. Horseradish peroxidase-linked anti-mouse IgG or anti-rabbit IgG (Cell Signaling) was used as a secondary antibody. Proteins were detected using an ImageQuant LAS4000 mini chemiluminescent image analyzer (GE Healthcare).

2.7. Real-time RT-PCR analysis

Intracellular and extracellular DENV RNA were extracted from infected cells using RNeasy minikit (QIAGEN) and from culture supernatants using QIAamp viral RNA minikit (QIAGEN), respectively. cDNA was synthesized from the isolated RNA using High Capacity RNA-to-cDNA kit (Life Technologies). Real-time PCR analysis of cDNA to detect DENV RNA was performed by previously described primers (d2C16A and d2C46B) and a fluorescent-labeled probe (VICd2C38B) targeting capsid gene of DENV genome [24] using TaqMan Gene Expression Master Mix (Life Technologies) and ABI Prism 7500 (Life Technologies). For quantification, serial dilutions of *in vitro* transcribed RNA containing DENV-2 capsid sequence were used to plot a standard curve.

2.8. ISGylation assay

Co-immunoprecipitation analysis to detect ISGylation of DENV protein was performed as described previously [25] with slight modifications. HEK293T cells (4×10^5 cells in 60 mm dish at 1 day prior transfection) were transfected with 2 μg of ISG15-pCAG-HA, 1 μg of Ube1L-pcDNA3, 1 μg of Ubch8-pcDNA3 and 2 μg of DENV protein expression plasmid using Lipofectamine 2000. Two days after transfection, cells were lysed in immunoprecipitation (IP) buffer (25 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodiumdeoxycholate, 0.1% SDS, 1 mM dithiothreitol, and protease inhibitors). After centrifugation to remove cell debris, lysates were cleared with protein G Sepharose beads (GE Healthcare) for 15 min, and the supernatant was incubated with 2 μg of anti-FLAG mouse monoclonal antibody (M2) for 3 h at 4 °C. Antibody-protein mixtures were then incubated for another 30 min with protein G Sepharose beads. The beads were washed three times with IP buffer, and total and ISG15-conjugated DENV proteins in immunoprecipitates were detected by immunoblotting analysis using anti-FLAG and anti-HA antibodies, respectively.

3. Results and discussion

As mentioned, a recent study reported that the silencing of endogenous ISG15 by siRNA in murine cells augmented DENV replication, indicating that ISG15 expression somehow acts to inhibit DENV infection [18]. However, the molecular details underlying the ISG15-mediated suppression of DENV, particularly in human cells, remains unknown. In order to clarify whether ISGylation is required for the ISG15-mediated anti-DENV effect in human cells,

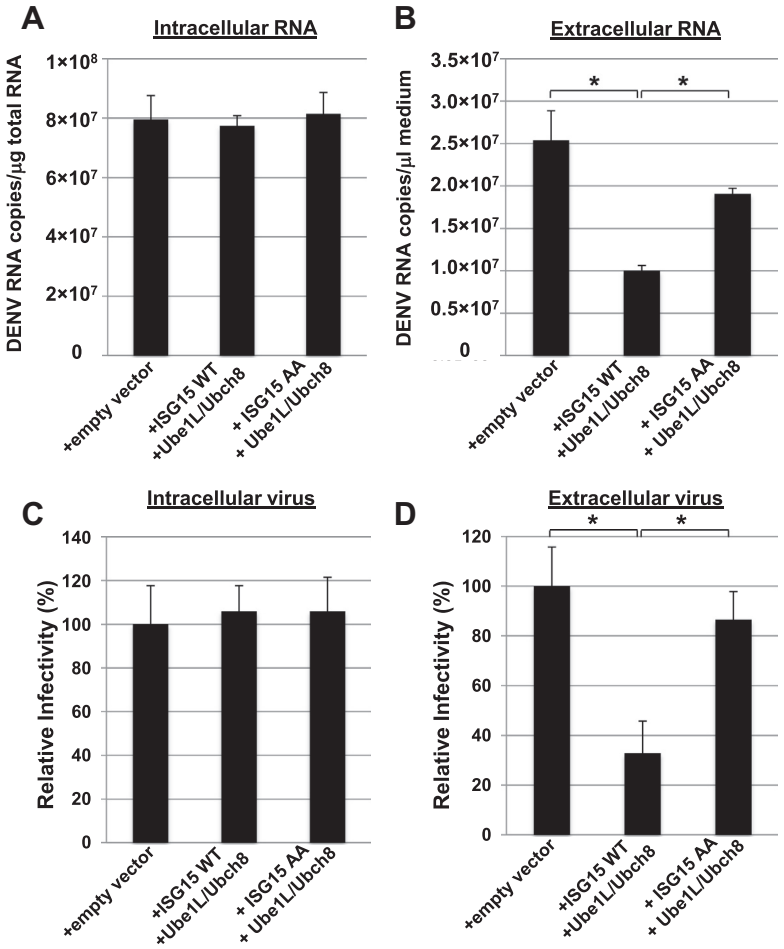

Fig. 1. ISGylation inhibits DENV-2 release. (A and B) Intracellular and extracellular levels of DENV RNA. HeLa cells were transfected with pCAG-HA-ISG15 (ISG15 WT) or pGAG-HA-ISG15AA mutant (ISG15 AA) in the presence of pcDNA3-Ube1L and pcDNA3-Ubch8. Empty vector plasmid pCAG was used as a negative control. Four hours after transfection, cells were inoculated with DENV-2 at MOI of 10. Amount of DENV RNA in the cell lysates (A) and culture supernatants (B) were analyzed by qRT-PCR using DENV-2 capsid sequence specific primers. (C and D) Intracellular and extracellular infectivity of DENV. Infectious DENV extracted from infected cells (C) and culture medium of the infected cells (D) were inoculated into BHK-21 cells, and infectious titers of DENV were quantified by plaque forming unit assay. In all the data, the average values of three independent experiments are shown, and the standard deviation (SD) of the means are presented. Statistical analyses were performed using the Student's *t*-test against cells expressing pCAG-HA-ISG15 (WT), and $P < 0.05$ (*) was considered statistically significant.

HeLa cells were transfected with plasmids expressing the ISGylation components, HA-tagged wild-type (WT) ISG15, Ube1L and Ubch8 [14], and then infected with DENV-2 at MOI of 10. When the amount of DENV RNA in infected cells was analyzed at 48 h after infection by qRT-PCR, comparable levels of intracellular viral RNA were detected in both mock- and ISGylation component-transfected cells (Fig. 1A). In contrast, a significantly lower amount of viral RNA was detected in the supernatant of infected HeLa cells transfected with ISGylation components containing WT HA-ISG15 when compared to mock-transfected cells (Fig. 1B, 60.6 ± 8.2% reduction). Interestingly, when a conjugation defective ISG15 mutant (containing a GG-to-AA substitution) HA-ISG15-AA was overexpressed [26], the significant reduction in extracellular viral RNA levels observed with WT HA-ISG15 was abolished (Fig. 1B). In addition, DENV infectivity assay revealed that the amount of intracellular infectious DENV-2 was unchanged amongst mock, WT ISG15 and conjugation-defective ISG15-transfected HeLa cells (Fig. 1C), whereas infectious virus titers in culture supernatant of the infected cells was significantly decreased to 67.2 ± 17.3% by the expression of WT ISG15 but not mutant ISG15 (Fig. 1D). This result suggests that ISG15 is able to inhibit DENV virion release in human cells, and ISGylation is required for the effect.

ISG15 is one of the most abundantly-induced genes of type-I IFN treatment [14]. A previous study has demonstrated that pretreatment of cells with IFN results in the inhibition of DENV replication [7]. We therefore examined if ISGylation could participate in the IFN-mediated anti-DENV functions. Endogenous ISG15 was depleted in HeLa cells by siRNA, and the level of inhibition of DENV replication by type-I IFN was analyzed. Expression levels of ISG15 were first verified by immunoblotting analysis. As expected, ISG15 expression was induced by type-I IFN (IFN-α/ω) treatment in control siRNA (si-control)-transfected cells (Fig. 2A). In contrast, transfection of ISG15-specific siRNA (si-ISG15) efficiently eliminated the IFN-induced ISG15 expression in HeLa cells (Fig. 2A). We next compared intracellular and extracellular levels of DENV-2 RNA in the IFN-treated cells by qRT-PCR analysis. Result showed that, as previously reported [7], IFN-α/ω treatment greatly decreased viral RNA levels in the cell lysate, demonstrating the type-I IFN-mediated antiviral state against DENV (Fig. 2B). Interestingly, although DENV RNA in the supernatant was also significantly reduced by IFN treatment, ISG15 knockdown increased the amount of extracellular viral RNA by 2.2-fold (Fig. 2C). This enhancement by ISG15 silencing was not observed in the absence of type-I IFN treatment (Fig. 2C). Given that ISGylation inhibited the release of DENV from infected cells (Fig. 1), and that depletion of endogenous ISG15 had no effect on the inhibition of intracellular viral RNA synthesis by IFN treatment (Fig. 2B), these results indicate that ISG15 substantially contributes to the IFN-mediated inhibition of DENV replication through suppression of virion release.

Previous studies on other RNA viruses have demonstrated that viral proteins can be targets of ISGylation [15–17]. To examine whether any of DENV proteins are subjected to ISGylation, co-immunoprecipitation assay using HA-ISG15 was performed. Although we have attempted to construct FLAG-tagged expression plasmids for all 10 DENV-2 proteins, E and NS2A proteins could not be expressed from the plasmid constructs. Thus the 8 other plas-

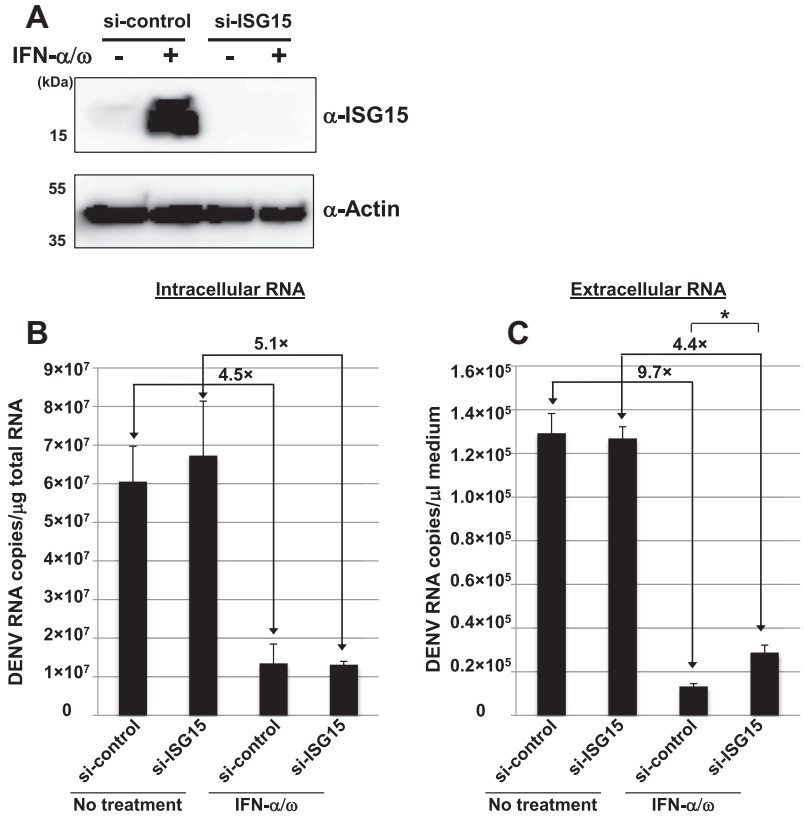

Fig. 2. ISG15 contributes to the IFN-mediated inhibition of DENV-2 release. (A) Depletion of endogenous ISG15 by siRNA transfection. HeLa cells were transfected with 50 nM siRNA duplex specific to human ISG15 mRNA or a control siRNA duplex (si-control). Four hours after transfection, cells were treated with 1000 units/ml type-I IFN for 8 h. Expression level of ISG15 and actin (internal control) were examined by immunoblotting analysis at 24 h after IFN treatment. (B and C) Effect of ISG15 knockdown on intracellular and extracellular levels of DENV-2 RNA. HeLa cells, which had been transfected with ISG15 siRNA and treated with type-I IFN, were inoculated with DENV-2 at a MOI of 1. Thirty-six hours after DENV infection, the amount of DENV RNA in infected cell lysates (B) and culture supernatants (C) were quantified by qRT-PCR. The average values of three independent experiments are shown and the standard deviations of the means are presented. *P* values were determined by Student's *t*-test comparison against cells expressing control siRNA; $^{*}P < 0.05$.

mid DNAs expressing FLAG-tagged C, prM, NS1, NS2B, NS3, NS4A, NS4B and NS5 proteins were used in the co-immunoprecipitation assay. DENV protein expression plasmids were individually co-transfected to HEK293T cells with HA-ISG15-, Ube1L- and Ubch8-expressing plasmids, and cell lysates were subjected to immunoprecipitation with anti-FLAG antibody. Immunoblotting analysis using anti-FLAG antibody showed that discrete high-molecular-mass bands, which did not correspond to the expected size of the FLAG-tagged NS proteins, were observed in immunoprecipitates from NS3- and NS5-transfected cells (Fig. 3, top panel). In contrast, such high-molecular-mass bands were not detected in immunoprecipitates from NS1- (Fig. 3, top panel), C-, prM-, NS2B-, NS4A-, or NS4B-expressing cells (Supplemental Fig. 1). This result implies that, at least amongst the DENV proteins tested, NS3 and NS5 appear to be subjected to post-translational modification(s). Importantly, a parallel immunoblotting analysis using anti-HA antibody to detect HA-ISG15 identified the high-molecular-mass bands of FLAG-NS3 and NS5 to be ISG15-conjugated proteins (Fig. 3, second panel). Therefore, this result indicates that DENV NS3 and NS5 undergo ISGylation in cells.

Treatment with IFN induces the expression of ISG15 and its conjugation enzymes in human cells, resulting in the ISGylation of several hundred cellular proteins [14]. The antiviral state established by ISG15 and ISGylation has been reported in various types of viruses, and overexpression of ISGylation components was indeed shown to hamper different processes of viral replication including virus particle release and viral protein stability [14–17]. In this study, we showed that ISGylation was likely to regulate DENV-2 replication through the suppression of virus releasing step. Supporting this, when ISGylation components were overexpressed in a stable cell line harboring DENV-2 RNA subgenomic replicon [20], the level of intracellular replicon RNA was not changed (data not shown). Nevertheless, a small but reproducible decrease in extracellular DENV RNA and infectivity was also observed by ISG15-AA overexpression (Fig. 1B and D), suggesting that unconjugated form of ISG15 might participate partly in the inhibition of DENV release with unknown mechanism of action.

Although the molecular mechanism underlying ISGylation-mediated suppression of DENV production remains unclear, our co-immunoprecipitation analysis using HA-tagged ISG15 revealed that NS3 and NS5 could be substrates for the ISGylation (Fig. 3). Of note, overall expression of DENV-2 NS3 and NS5 was unchanged by the transfection of ISGylation components (Fig. 3, third panel), indicating that conjugation of ISG15 probably does not influence the stability of the DENV NS proteins. NS3 and NS5 are multifunctional proteins possessing two distinct enzymatic activities on respective N- and C-terminal domains (i.e. protease and helicase domains in NS3 and methyltransferase and RNA-dependent RNA polymerase domains in NS5) and collaborate to play a central role in the functional DENV replication complex [3]. Intriguingly, some of flavivirus NS proteins have been demonstrated to be involved in virion assembly [27]. However, overexpression of ISGylation

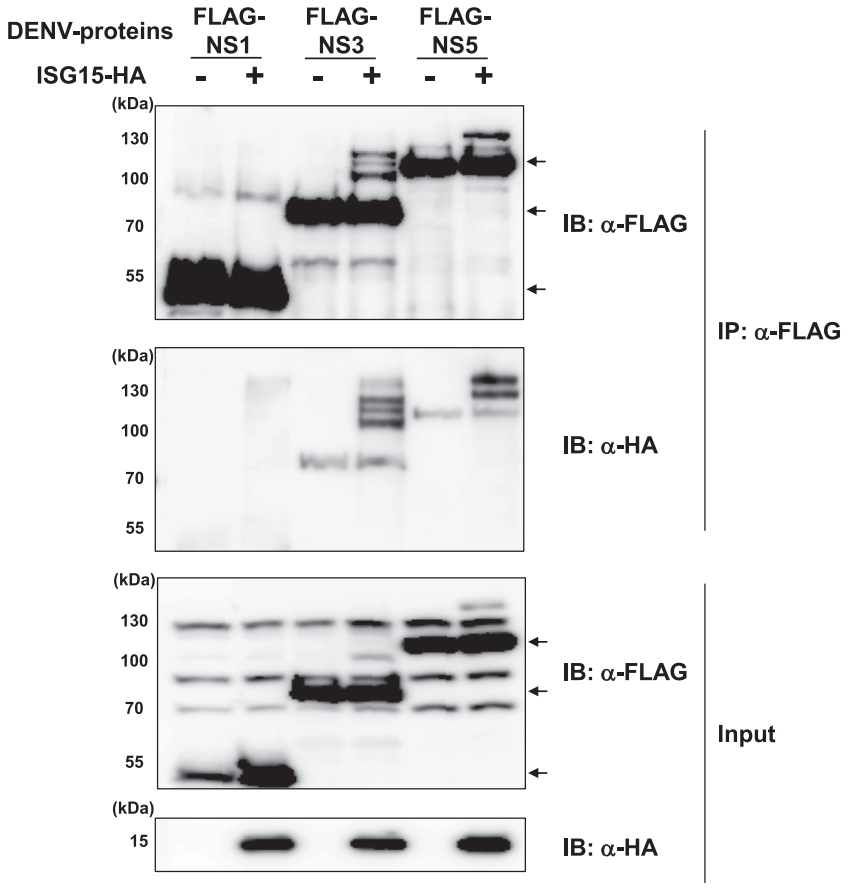

Fig. 3. DENV-2 NS3 and NS5 proteins undergo ISGylation. HEK293T cells were transfected with pCAG-FLAG-NS1, -NS3 or -NS5 in the presence (+) or absence (−) of plasmid DNAs expressing ISGylation components (pCAG-HA-ISG15, pcDNA3-Ube1L and pcDNA3-Ubch8). Forty-eight hours after transfection, proteins were immunoprecipitated from cell lysates with an anti-FLAG antibody (IP). Co-immunoprecipitated proteins were resolved by SDS–PAGE and analyzed by immunoblotting (IB) using anti-FLAG (top panel) and anti-HA (second panel) antibodies to detect DENV NS proteins and ISG15-conjugated proteins, respectively. FLAG-tagged DENV proteins (third panel) and HA-tagged ISG15 (bottom panel) in input fractions were also detected by immunoblotting analysis. Arrows indicate unconjugated DENV proteins. Molecular masses of standards are indicated on the left of each panel.

components did not decrease the infectivity of intracellular viruses, suggesting that ISGylation does not influence DENV assembly process (Fig. 1D). To our knowledge, while there is no report of involvement of NS3 and NS5 in DENV release step, extracellular release of Japanese encephalitis virus (JEV) E protein is reported to be enhanced by JEV NS3 [28]. It will therefore be intriguing in future to investigate if the NS3 and NS5 would act to facilitate virus release step during DENV replication and whether those functions could be impaired by ISGylation. Another likely possibility would be that subcellular localization of NS5, which predominantly localizes in nucleus [29], may be modulated by ISGylation, thereby transcriptionally downregulating the expression of host factors involved in the DENV release process. Since effective drug against DENV is not currently available, further studies to clarify the unknown mechanism by which ISGylation hampers DENV production will lead to the development of new treatments for DENV infection.

Acknowledgments

We are grateful to members of laboratory for helpful comments and discussions and also thank to Wei Xin Chin for proofreading on the manuscript. This work was supported by NUS SoM Start-up Grant (R-182-000-160-733, R-182-000-160-133) to N.Y.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.081.

References

[1] M.G. Guzman, S.B. Halstead, H. Artsob, P. Buchy, J. Farrar, D.J. Gubler, E. Hunsperger, A. Kroeger, H.S. Margolis, E. Martinez, M.B. Nathan, J.L. Pelegrino, C. Simmons, S. Yoksan, R.W. Peeling, Dengue: a continuing global threat, Nat. Rev. Microbiol. 8 (2010) S7–S16.
[2] D.W. Vaughn, S. Green, S. Kalayanarooj, B.L. Innis, S. Nimmannitya, S. Suntayakorn, T.P. Endy, B. Raengsakulrach, A.L. Rothman, F.A. Ennis, A. Nisalak, Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity, J. Infect. Dis. 181 (2000) 2–9.
[3] B.D. Lindenbach, C.M. Rice, Molecular biology of flaviviruses, Adv. Virus Res. 59 (2003) 23–61.
[4] E. Navarro-Sanchez, P. Despres, L. Cedillo-Barron, Innate immune responses to dengue virus, Arch. Med. Res. 36 (2005) 425–435.
[5] R.E. Randall, S. Goodbourn, Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures, J. Gen. Virol. 89 (2008) 1–47.
[6] J. Morrison, S. Aguirre, A. Fernandez-Sesma, Innate immunity evasion by Dengue virus, Viruses 4 (2012) 397–413.
[7] M.S. Diamond, T.G. Roberts, D. Edgil, B. Lu, J. Ernst, E. Harris, Modulation of Dengue virus infection in human cells by alpha, beta, and gamma interferons, J. Virol. 74 (2000) 4957–4966.
[8] K. Clyde, J.L. Kyle, E. Harris, Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis, J. Virol. 80 (2006) 11418–11431.
[9] A.J. Johnson, J.T. Roehrig, New mouse model for dengue virus vaccine testing, J. Virol. 73 (1999) 783–786.
[10] J. Ashour, J. Morrison, M. Laurent-Rolle, A. Belicha-Villanueva, C.R. Plumlee, D. Bernal-Rubio, K.L. Williams, E. Harris, A. Fernandez-Sesma, C. Schindler, A. Garcia-Sastre, Mouse STAT2 restricts early dengue virus replication, Cell Host Microbe 8 (2010) 410–421.
[11] G.C. Sen, Viruses and interferons, Annu. Rev. Microbiol. 55 (2001) 255–281.
[12] A.L. Brass, I.C. Huang, Y. Benita, S.P. John, M.N. Krishnan, E.M. Feeley, B.J. Ryan, J.L. Weyer, L. van der Weyden, E. Fikrig, D.J. Adams, R.J. Xavier, M. Farzan, S.J. Elledge, The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus, Cell 139 (2009) 1243–1254.
[13] D. Jiang, J.M. Weidner, M. Qing, X.B. Pan, H. Guo, C. Xu, X. Zhang, A. Birk, J. Chang, P.Y. Shi, T.M. Block, J.T. Guo, Identification of five interferon-induced cellular proteins that inhibit West Nile virus and dengue virus infections, J. Virol. 84 (2010) 8332–8341.
[14] Y.J. Jeon, H.M. Yoo, C.H. Chung, ISG15 and immune diseases, Biochim. Biophys. Acta 1802 (2010) 485–496.
[15] M.J. Kim, J.Y. Yoo, Inhibition of hepatitis C virus replication by IFN-mediated ISGylation of HCV-NS5A, J. Immunol. 185 (2010) 4311–4318.
[16] M.W. Woods, J.N. Kelly, C.J. Hattlmann, J.G. Tong, L.S. Xu, M.D. Coleman, G.R. Quest, J.R. Smiley, S.D. Barr, Human HERC5 restricts an early stage of HIV-1 assembly by a mechanism correlating with the ISGylation of Gag, Retrovirology 8 (2011) 95.
[17] C. Zhao, T.Y. Hsiang, R.L. Kuo, R.M. Krug, ISG15 conjugation system targets the viral NS1 protein in influenza A virus-infected cells, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 2253–2258.
[18] J. Dai, W. Pan, P. Wang, ISG15 facilitates cellular antiviral response to dengue and West Nile virus infection in vitro, Virol. J. 8 (2011) 468.
[19] J.G. Low, E.E. Ooi, T. Tolfvenstam, Y.S. Leo, M.L. Hibberd, L.C. Ng, Y.L. Lai, G.S. Yap, C.S. Li, S.G. Vasudevan, A. Ong, Early Dengue infection and outcome study (EDEN) - study design and preliminary findings, Ann. Acad. Med. Singapore 35 (2006) 783–789.
[20] C.Y. Ng, F. Gu, W.Y. Phong, Y.L. Chen, S.P. Lim, A. Davidson, S.G. Vasudevan, Construction and characterization of a stable subgenomic dengue virus type 2 replicon system for antiviral compound and siRNA testing, Antiviral Res. 76 (2007) 222–231.
[21] K. Arimoto, H. Konishi, K. Shimotohno, UbcH8 regulates ubiquitin and ISG15 conjugation to RIG-I, Mol. Immunol. 45 (2008) 1078–1084.
[22] L. Gama, G.E. Breitwieser, Generation of epitope-tagged proteins by inverse PCR mutagenesis, Biotechniques 26 (1999) 814–816.
[23] T. Hishiki, Y. Shimizu, R. Tobita, K. Sugiyama, K. Ogawa, K. Funami, Y. Ohsaki, T. Fujimoto, H. Takaku, T. Wakita, T.F. Baumert, Y. Miyanari, K. Shimotohno, Infectivity of hepatitis C virus is influenced by association with apolipoprotein E isoforms, J. Virol. 84 (2010) 12048–12057.
[24] W.K. Wang, T.L. Sung, Y.C. Tsai, C.L. Kao, S.M. Chang, C.C. King, Detection of dengue virus replication in peripheral blood mononuclear cells from dengue virus type 2-infected patients by a reverse transcription-real-time PCR assay, J. Clin. Microbiol. 40 (2002) 4472–4478.
[25] T. Hishiki, T. Ohshima, T. Ego, K. Shimotohno, BCL3 acts as a negative regulator of transcription from the human T-cell leukemia virus type 1 long terminal repeat through interactions with TORC3, J. Biol. Chem. 282 (2007) 28335–28343.
[26] D.J. Lenschow, N.V. Giannakopoulos, L.J. Gunn, C. Johnston, A.K. O'Guin, R.E. Schmidt, B. Levine, H.W.t. Virgin, Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo, J. Virol. 79 (2005) 13974–13983.
[27] C.L. Murray, C.T. Jones, C.M. Rice, Architects of assembly: roles of Flaviviridae non-structural proteins in virion morphogenesis, Nat. Rev. Microbiol. 6 (2008) 699–708.
[28] T. Sato, C. Takamura, A. Yasuda, M. Miyamoto, K. Kamogawa, K. Yasui, High-level expression of the Japanese encephalitis virus E protein by recombinant vaccinia virus and enhancement of its extracellular release by the NS3 gene product, Virology 192 (1993) 483–490.
[29] M. Kapoor, L. Zhang, M. Ramachandra, J. Kusukawa, K.E. Ebner, R. Padmanabhan, Association between NS3 and NS5 proteins of dengue virus type 2 in the putative RNA replicase is linked to differential phosphorylation of NS5, J. Biol. Chem. 270 (1995) 19100–19106.