Depleted Immunoglobulin Heavy Chain Binding Protein (BiP) Expands the Endoplasmic Reticulum and the Golgi Apparatus in Dengue Virus-Infected Cells

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ABSTRACT

Objective: To test whether depleted expression of immunoglobulin heavy chain binding protein (BiP), which is an endoplasmic reticulum (ER) chaperone, in dengue virus (DENV)-infected cells, affected integrities of the ER and the Golgi apparatus of the host cells.

Methods: Either siRNA against BiP- or control siRNA-transfected cells was infected with DENV and subjected to electron microscopic evaluation.

Results: Depleted expression of BiP affected integrities of the ER and the Golgi apparatus in DENV-infected cells.

Conclusion: Integrities of the ER and the Golgi apparatus maintained by BiP in the host cells is necessary for DENV production.

Keywords: Dengue virus, BiP, Endoplasmic reticulum, Golgi apparatus, RNA interference, electron microscopy

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INTRODUCTION

Dengue virus (DENV) infection is an important mosquito-borne disease and a public health problem worldwide. Clinical severity ranges from febrile dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DENV belongs to the *Flaviviridae* family and contains a single positive-stranded RNA genome, encoding a single precursor polypeptide. Host and viral proteases cleave this polypeptide into three structural proteins (DENV C, DENV E and DENV prM) and seven nonstructural proteins (DENV NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5), respectively. Currently, there are no effective agents or proved vaccines for the DENV infection.

Replication of flaviviruses occurs in association with the ER where virions assemble and bud into the lumen of the ER. Virus particles transit through trans-Golgi network (TGN) where they undergo the maturation prior to being released by exocytosis. Defects in the mechanisms controlling the proper protein folding and the assembly mediated by the ER and the Golgi apparatus affect morphogenesis and production of virions.[1-4] We previously showed that DENV envelope protein (DENV E) interacts with human immunoglobulin heavy chain binding protein (BiP), demonstrated by co-immunoprecipitation and co-localization of BiP and DENV E in DENV-infected cells. In addition, depleted expression of BiP by siRNA significantly decreased the production of infectious DENV virions.[5,6]

In this study, we demonstrate that, depleted expression of BiP affected integrities of the ER and the Golgi apparatus in DENV-infected cells thereby affecting DENV production.

MATERIALS AND METHODS

Knockdown of BiP in DENV-infected cells

The BiP siRNA (5' GCGGAACCTTCGATGTGTCTCTTCT 3') was purchased from Invitrogen, USA and used to knock-down BiP by transfection into Vero cells using LipofectamineTM 2000 reagent (Invitrogen, USA). Transfection with irrelevant siRNA (Invitrogen, USA, Cat. No. 12935-300) was performed as a negative control. After 6 h of transfection, cells were fed with 10% FBS in MEM medium for 30 h. Then, siRNA-transfected cells were infected with DENV serotype 2 strain 16681 at an MOI of 1 for 3 h. Samples were taken for mRNA and protein analysis using real-time PCR (Lightcycler

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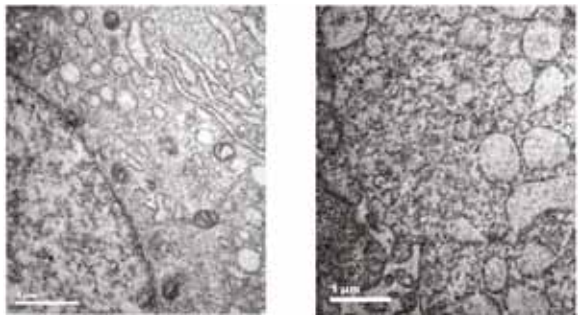

A B

Fig 1. TEM of DENV-infected siRNA-transfected cells
Either siBiP- or control siRNA-transfected cells was infected with DENV. After transfection, the cells were prepared for electron microscopic evaluation. DENV-infected cells transfected with control siRNA showed normally distributed sub-cellular organelles such as the ER and the Golgi apparatus (Fig 1A) but there was a dramatic change of sub-cellular organelles, especially in the ER and the Golgi apparatus, in DENV-infected cells transfected with siRNA against BiP (Fig 1B).

RNA amplification kit, Roche) and Western blot analysis, respectively.[5] The DENV infected siRNA-transfected cells were washed with PBS and fed with 2% FBS in MEM medium for 24 h.

Transmission electron microscopy of DENV-infected siRNA-transfected cells

The DENV infected siRNA-transfected cells were harvested and washed with phosphate buffered saline (PBS). Consequently, the cell samples were fixed with 2.5% glutaraldehyde in PBS and postfixed with 1% osmium tetroxide. Next, the cells were dehydrated by serial increments through ethanol and then treated with propylene oxide prior to being embedded in resin blocks. Ultrathin (90 nm) sections were cut by an ultramicrotome (Lieca EM UC6, Vienna, Austria) and stained with 1% uranyl acetate and lead citrate. The sections were viewed using the transmission electron microscope (JEOL JEM 100S, Tokyo, Japan).

RESULTS

Depleted expression of BiP by siRNA significantly decreased the production of infectious DENV virions.[5,6] To test whether depleted expression of BiP in DENV-infected cells affected integrities of the ER and the Golgi apparatus of the host cells, either siBiP- or control siRNA-transfected cells was infected with DENV. After transfection, the cells were prepared for transmission electron microscopic evaluation. As shown in Fig 1A, DENV-infected cell transfected with control siRNA showed normally distributed sub-cellular organelles. The cytoplasm of DENV-infected cells transfected with control siRNA contained the ER, and Golgi apparatus suggesting for the ability of the cell to fold a glycoprotein in the ER and to sort a protein in the trans-Golgi network (TGN), respectively. However, in BiP depleted cells, there was a dramatic change of sub-cellular organelles, especially in the ER and Golgi apparatus, observed by the extreme expansions of ER and Golgi apparatus, which reflected the defective abilities of the ER to properly fold a glycoprotein, and the Golgi apparatus to correctly sort a cargo protein in BiP depleted cells (Fig 1B) when compared to the DENV-infected cells transfected with control siRNA (Fig 1A).

DISCUSSION

The glycosylated DENV E is a cargo protein that is processed in the the ER of the host cells and therefore reliant on host processing functions. Exocytic cargo protein from ER to Golgi apparatus is believed to travel within the Golgi apparatus by intercisternal vesicular transport and then to exit the TGN in vesicular carriers and consumed by a series of budding and fusion steps. We previously reported the physical interaction between DENV E and the ER-resident chaperone, namely BiP, and its contribution in the production of DENV infectious particles.[5] Recently, Wati S et al, also demonstrated that the cleavage of BiP with the SubAB toxin during DENV infection resulted in a 10- to 100-fold decrease in infectious-virus release, a loss of intracellular DENV particles, and a dramatic decrease in intracellular DENV antigen.[6] We further showed in this study for the detailed molecular mechanisms how DENV assemble and egress by RNA interference and TEM evaluation. The result implicates a role of BiP in DENV production as in BiP depleted cells, there was a dramatic change of sub-cellular organelles, especially in the ER and the Golgi apparatus, observed by the extreme expansions of ER and Golgi apparatus, which reflected the defective ability of ER to properly fold a glycoprotein, and the Golgi apparatus to correctly sort DENV E in BiP depleted cells (Fig 1B) when compared to DENV-infected cells transfected with control siRNA (Fig 1A).

CONCLUSION

BiP is essential for DENV production as depleted expression of BiP affects the integrities of the ER and the Golgi apparatus in the host cells thereby decreasing the production of DENV virions.

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