Antiviral Research 109 (2014) 171–174

Contents lists available at ScienceDirect

Antiviral Research

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

Short Communication

HSPA5 is an essential host factor for Ebola virus infection

St. Patrick Reid, Amy C. Shurtleff, Julie A. Costantino [1], Sarah R. Tritsch, Cary Retterer, Kevin B. Spurgers [2], Sina Bavari *

United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011, USA

ARTICLE INFO

Article history:
Received 14 May 2014
Revised 1 July 2014
Accepted 3 July 2014
Available online 11 July 2014

Keywords:
Ebolavirus
HSPA5
Antiviral

ABSTRACT

Development of novel strategies targeting the highly virulent ebolaviruses is urgently required. A proteomic study identified the ER chaperone HSPA5 as an ebolavirus-associated host protein. Here, we show using the HSPA5 inhibitor (-)- epigallocatechin gallate (EGCG) that the chaperone is essential for virus infection, thereby demonstrating a functional significance for the association. Furthermore, *in vitro* and *in vivo* gene targeting impaired viral replication and protected animals in a lethal infection model. These findings demonstrate that HSPA5 is vital for replication and can serve as a viable target for the design of host-based countermeasures.

Published by Elsevier B.V.

Productive Ebola virus (EBOV) infection requires successful recruitment of host factors for the various stages of the viral life cycle. There are currently no approved therapeutic strategies for treating infection, therefore the absolute dependence on these factors, owing in large part to the limited number of viral gene products offers a promising area for therapeutic intervention. Toward this end, we have identified the endoplasmic reticulum (ER) chaperone, heat shock 70 kDa protein 5 (HSPA5) as an EBOV-associated host factor (Spurgers et al., 2010).

HSPA5 is a highly conserved ER resident protein involved in the folding and assembly of nascent proteins. The chaperone also serves as master regulator of ER stress responses (Hendershot, 2004; Lee, 2005). In addition to host protein chaperone function, HSPA5 has also been demonstrated to play a key role during viral infections (Mayer, 2005). The chaperone function of HSPA5 is instrumental in the maturation of envelope proteins from a number of viruses including, Sindbis virus (SINV), hepatitis C virus (HCV), vesicular stomatitis virus (VSV) and influenza A virus (de Silva et al., 1990; Machamer et al., 1990; Singh et al., 1990; Hogue and Nayak, 1992; Mulvey and Brown, 1995; Choukhi et al., 1998). Departing from a role in protein folding, novel functions for HSPA5 have also been described during infection. Notably, for viruses such as coxsackievirus A9 (CVA9), Borna disease virus (BDV) and dengue virus serotype 2 (DENV2), HSPA5 plays a role in viral entry (Triantafilou et al., 2002; Jindadamrongwech et al., 2004; Honda et al., 2009).

Association of HSPA5 with EBOV suggests that it may be essential for infection. To determine this, we utilized the small molecule (-)- epigallocatechin gallate (EGCG). EGCG binds the ATP-binding site of HSPA5 inhibiting its ability to bind ATP (Ermakova et al., 2006). This results in inhibition of HSPA5 ATPase activity, a critical function for chaperone proteins. HeLa cells were treated with increasing concentrations of EGCG (10–100 μM) for 2 h then infected with EBOV. Forty-eight hours post infection the cells were fixed and stained for EBOV infection. EGCG consistently exhibited a dose dependent inhibition of EBOV infection (Fig. 1A and B), indicating that HSPA5 ATPase activity and therefore chaperone function is, at least in part, required for EBOV infection.

Modulation of HSPA5 expression was previously reported to affect DENV protein production (Wati et al., 2009). To investigate whether HSPA5 plays a similar role during EBOV infection, 293T cells were transfected with a non-target or HSPA5 siRNA. Forty-eight hours post transfection the cells were infected and cell lysates collected 24 or 48 h post infection. Transfecting cells with the non-target siRNA had little effect on viral transcript production (Fig. 2A). In contrast, transfection of the HSPA5 siRNA resulted in a significant decrease in viral transcript production at 24 h and to a greater extent at 48 h post infection (Fig. 2A). Correspondingly, VP24 protein production was significantly reduced at 48 h post infection (Fig. 2B), indicating that HSPA5 is essential for the production of EBOV transcripts and proteins. Knockdown of HSPA5 was confirmed in both infected and uninfected cell lysates (Fig. 2C). Interestingly, while modest at 24 h, at 48 h we observed

* Corresponding author.
E-mail address: sina.bavari@amedd.army.mil (S. Bavari).
[1] Current address: Lonza, 8830 Biggs Ford Rd, Walkersville, MD, 21793, USA.
[2] Current address: Booz Allen Hamilton, 8283 Greensboro Drive, McLean, VA, 22102, USA.

http://dx.doi.org/10.1016/j.antiviral.2014.07.004
0166-3542/Published by Elsevier B.V.

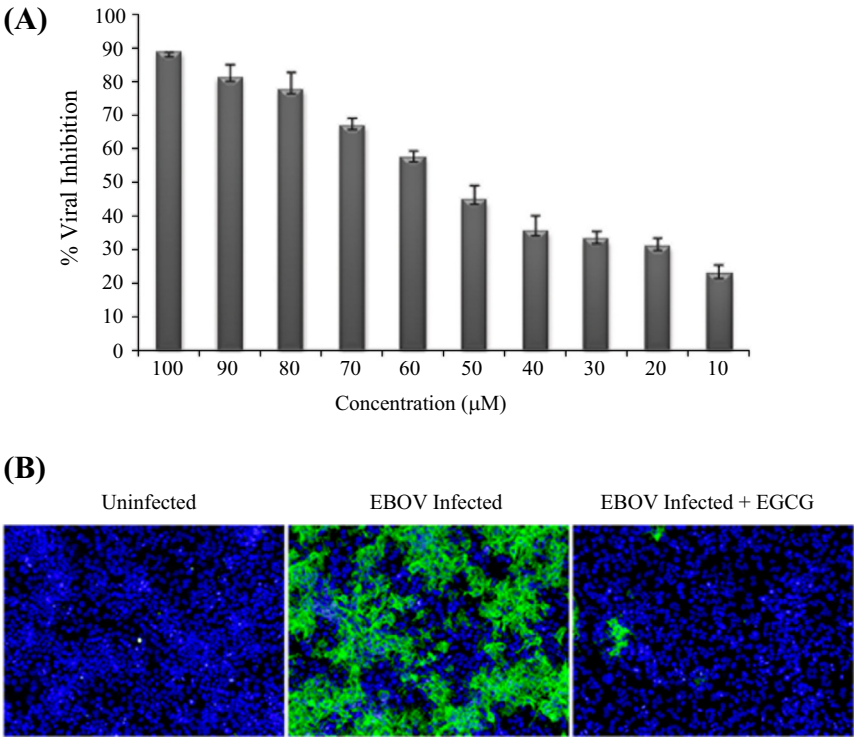

Fig. 1. EGCG pre-treatment inhibits EBOV infection. (A) HeLa cells were treated with the indicated concentration of EGCG for 2 h then infected with EBOV at an MOI of 5. Forty-eight hours post infection the cells were fixed and processed for immunofluorescence detection of viral antigen. (B) Representative images from the automated fluorescence analysis are shown. In the EBOV infected + EGCG panel the 100 μM concentration of EGCG is shown. Infected cells were detected with a mouse antibody against EBOV $GP_{1,2}$ and an Alexa fluor-488 secondary antibody. Cell nuclei (blue) were stained with Hoechst 33342.

an increase in HSPA5 transcript levels in untransfected infected compared to uninfected samples and similarly, in non-target infected compared to uninfected samples (Fig. 2C), suggesting EBOV infection upregulates HSPA5 expression. A potential mechanism for this includes EBOV $GP_{1,2}$ accumulation in the ER, which is thought to be associated with an ER stress response (Bhattacharyya and Hope, 2011). Taken together, HSPA5 expression is increased during EBOV infection and is required for transcription production.

HSPA5 has been described to play the novel role as an entry factor during infection (Triantafilou et al., 2002; Jindadamrongwech et al., 2004; Honda et al., 2009). We were therefore interested in investigating a potential role in EBOV entry and egress. Knockdown studies using VSV pseudotyped with EBOV $GP_{1,2}$ did not show an effect of HSPA5 on EBOV entry (data not shown). We next examined viral egress by monitoring release of VP40 from cells in the presence or absence of HSPA5 knockdown. 293T cells were left untransfected or transfected with a non-target or HSPA5 siRNA. Two days post-transfection the cells were transfected with a VP40 expression plasmid. Two days after, cell supernatants were harvested and VLPs isolated by centrifugation through a 20% sucrose cushion, and the cellular material lysed. Reproducibly in these assays VP40 expression in cell lysates was found to be similar in all three conditions; in contrast, VP40 VLP levels were significantly reduced in HSPA5 siRNA treated cells (Fig. 2D). These data suggest that HSPA5 plays a novel role in VP40 budding. It should be noted that an interaction between VP40 and HSPA5 has been suggested (Yamayoshi et al., 2008), however, in these studies we were unable to detect this interaction, although an interaction with $GP_{1,2}$ was observed (data not shown). Nonetheless one can speculate that HSPA5 involvement in virus budding is a way in which the chaperone protein becomes associated with EBOV.

Phosphorodiamidate morpholino oligomers (PMOs) are a class of antisense DNA nucleotide analogs that have shown promise in studies targeting viral infection (Reid et al., 2012; Warren et al., 2012). In particular, *in vivo* efficacy has been demonstrated for PMOs targeting filovirus transcripts (Enterlein et al., 2006; Warfield et al., 2006; Warren et al., 2010; Iversen et al., 2012). Based on our *in vitro* findings we wanted to determine whether PMOs targeting HSPA5 *in vivo* could protect mice from lethal EBOV challenge. Groups of 10 C57BL/6 mice were treated intraperitoneally (i.p.) 24 and 1 h prior to infection and again at days 1 and 4 post-infection with PBS, a control PMO, or a PMO targeting HSPA5 (HSPA5–PMO), at a dose of 7.5 mg/kg (Fig. 3A). Mice were challenged with 1000 PFU EBOV and monitored for survival. Consistent with previous findings we observed a high degree of mortality (100%) in mice treated with either PBS or scrambled PMO controls (Fig. 3B). In contrast, mice treated with the HSPA5–PMO were completely protected from lethal EBOV challenge (Fig. 3B). These data further support a critical role for HSPA5 during EBOV infection. It is worth noting that prior EBOV PMO studies exclusively targeted viral transcripts; therefore to our knowledge this is the first successful *in vivo* use of a host-based gene targeting approach against EBOV. Targeting HSPA5 has an advantage over direct targeting of viral gene products, since the dependence on these factors makes development of escape mutants more difficult. Additionally, there is greater potential to develop a broad spectrum therapeutic. In support of this, preliminary studies indicate targeting HSPA5 also protects against marburvirus infection (data not shown). These animal studies were performed in a biosafety level 4 (BSL-4) laboratory at the U.S. Army Medical Research Institute of Infectious Diseases, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the 8th edition of the Guide

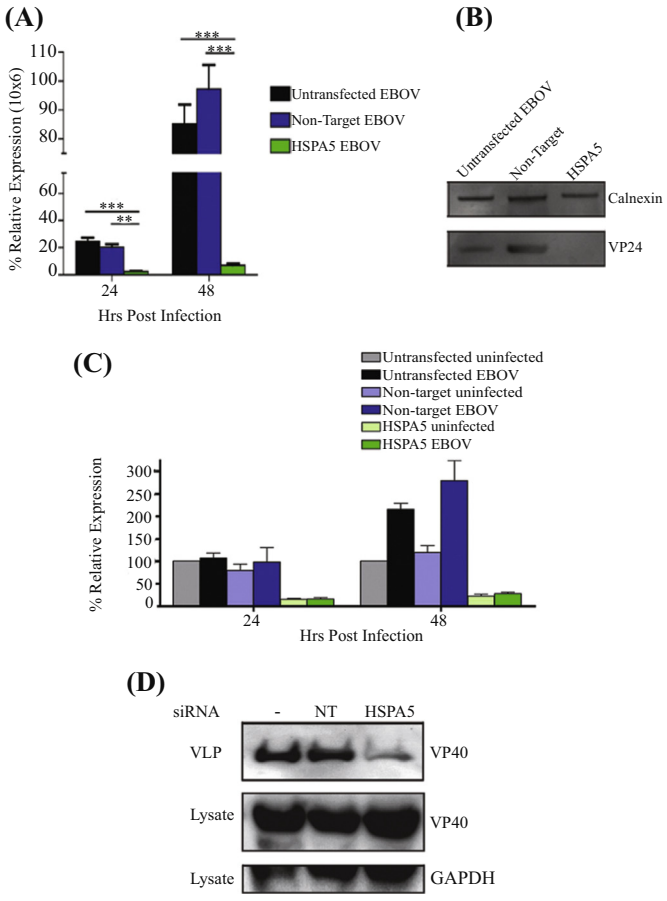

Fig. 2. Knockdown of HSPA5 inhibits EBOV replication and VP40 budding. (A) 293T cells were left untreated, treated with a non-target siRNA or an HSPA5 siRNA for 48 h then either mock infected or infected with EBOV at an MOI of 5 for 24 or 48 h. RNA was isolated by Trizol extraction and analyzed for the presences viral (A) or HSPA5 (C) transcript by qRT–PCR. (B) Forty-eight hours infected cell lysates obtained from Trizol extraction were separated by SDS–PAGE and analyzed by western blotting with anti-VP24 and anti-calnexin antibodies. (D) 293T cells were left untransfected, transfected with non-target siRNA or an HSPA5 siRNA. Forty-eight hours post transfection cells were transfected with an EBOV VP40 expression plasmid. VP40 VLPs were isolated from the culture medium by centrifugation through a 20% sucrose cushion 48 h post plasmid transfection. Isolated VP40 VLPs and cell lysate were separated by SDS–PAGE and analyzed by western blotting. $^{**}p < 0.01$; $^{***}p < 0.001$ values were determined using one way ANOVA with Bonferroni's multiple correction test.

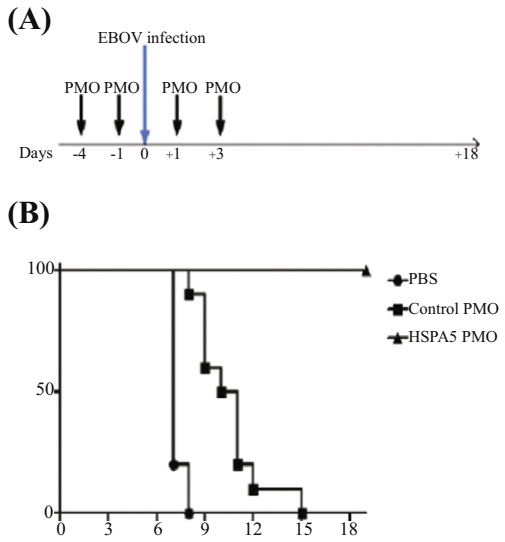

Fig. 3. PMOs targeting HSPA5 protect C57BL/6 mice against lethal EBOV infection. (A) A schematic illustration of the PMO treatment and EBOV challenge schedule. (B) Groups of mice (n = 10) were treated as indicated by i.p. injection with 7.5 mg/kg dose and animal health and survival were monitored for up to 18.

for the Care and Use of Laboratory Animals, National Council, 2011. The research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy and other Federal statutes and regulation relating to animal and experiments involving animals.

In the current study we extend upon our previous identification of HSPA5 as an EBOV-associated host factor, and demonstrate that it is essential for EBOV infection. Targeting HSPA5 both *in vitro* and *in vivo* resulted in significant reduction in virus replication and protection of mice against lethal virus challenge, respectively. Interestingly, a surrogate model for studying virus release using ectopically expressed VP40 indicated that HSPA5 is also required for budding (Fig. 2D). Therefore we have identified a critical host factor for EBOV infection. Based on the current findings in this report we propose that HSPA5 is viable target for the development of anti-filovirus countermeasures.

Acknowledgements

We thank Sean Van Tongeren for performing mouse infections and Keren Rabinowitz for technical assistance. The research described herein was sponsored by the Defense Threat Reduction Agency (JSTO-CBD project number 44.10022-08-RD-B and TMTI

project number 0048-09-RD-T). The opinions, interpretation, conclusions and recommendations in this report are not necessarily endorsed by the U.S. Army.

References

Bhattacharyya, S., Hope, T.J., 2011. Full-length Ebola glycoprotein accumulates in the endoplasmic reticulum. J. Virol. 8, 11.
Choukhi, A., Ung, S., et al., 1998. Involvement of endoplasmic reticulum chaperones in the folding of hepatitis C virus glycoproteins. J. Virol. 72 (5), 3851–3858.
de Silva, A.M., Balch, W.E., et al., 1990. Quality control in the endoplasmic reticulum: folding and misfolding of vesicular stomatitis virus G protein in cells and in vitro. J. Cell Biol. 111 (3), 857–866.
Enterlein, S., Warfield, K.L., et al., 2006. VP35 knockdown inhibits Ebola virus amplification and protects against lethal infection in mice. Antimicrob. Agents Chemother. 50 (3), 984–993.
Ermakova, S.P., Kang, B.S., et al., 2006. (-)-Epigallocatechin gallate overcomes resistance to etoposide-induced cell death by targeting the molecular chaperone glucose-regulated protein 78. Cancer Res. 66 (18), 9260–9269.
Hendershot, L.M., 2004. The ER chaperone BiP is a master regulator of ER function. Mt. Sinai J. Med. 71 (5), 289–297.
Hogue, B.G., Nayak, D.P., 1992. Synthesis and processing of the influenza virus neuraminidase, a type II transmembrane glycoprotein. Virology 188 (2), 510–517.
Honda, T., Horie, M., et al., 2009. Molecular chaperone BiP interacts with Borna disease virus glycoprotein at the cell surface. J. Virol. 83 (23), 12622–12625.
Iversen, P.L., Warren, T.K., et al., 2012. Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. Viruses 4 (11), 2806–2830.
Jindadamrongwech, S., Thepparit, C., et al., 2004. Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. Arch. Virol. 149 (5), 915–927.
Lee, A.S., 2005. The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. Methods 35 (4), 373–381.
Machamer, C.E., Doms, R.W., et al., 1990. Heavy chain binding protein recognizes incompletely disulfide-bonded forms of vesicular stomatitis virus G protein. J. Biol. Chem. 265 (12), 6879–6883.
Mayer, M.P., 2005. Recruitment of Hsp70 chaperones: a crucial part of viral survival strategies. Rev. Physiol. Biochem. Pharmacol. 153, 1–46.
Mulvey, M., Brown, D.T., 1995. Involvement of the molecular chaperone BiP in maturation of Sindbis virus envelope glycoproteins. J. Virol. 69 (3), 1621–1627.
Reid, S., Shurtleff, A.C., et al., 2012. Emerging therapeutic options against filoviruses. Drugs of the Future 37 (5), 343–351.
Singh, I., Doms, R.W., et al., 1990. Intracellular transport of soluble and membrane-bound glycoproteins: folding, assembly and secretion of anchor-free influenza hemagglutinin. EMBO J. 9 (3), 631–639.
Spurgers, K.B., Alefantis, T., et al., 2010. Identification of essential filovirion-associated host factors by serial proteomic analysis and RNAi screen. Mol. Cell. Proteomics 9 (12), 2690–2703.
Triantafilou, K., Fradelizi, D., et al., 2002. GRP78, a coreceptor for coxsackievirus A9, interacts with major histocompatibility complex class I molecules which mediate virus internalization. J. Virol. 76 (2), 633–643.
Warfield, K.L., Swenson, D.L., et al., 2006. Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers. PLoS Pathog. 2 (1), e1.
Warren, T.K., Shurtleff, A.C., et al., 2012. Advanced morpholino oligomers: a novel approach to antiviral therapy. Antivir. Res. 94 (1), 80–88.
Warren, T.K., Warfield, K.L., et al., 2010. Advanced antisense therapies for postexposure protection against lethal filovirus infections. Nat. Med. 16 (9), 991–994.
Wati, S., Soo, M.L., et al., 2009. Dengue virus infection induces upregulation of GRP78, which acts to chaperone viral antigen production. J. Virol. 83 (24), 12871–12880.
Yamayoshi, S., Noda, T., et al., 2008. Ebola virus matrix protein VP40 uses the COPII transport system for its intracellular transport. Cell Host Microbe 3 (3), 168–177.