Hypusination of eIF5A as a Target for Antiviral Therapy

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VIRUSES ARE A RECURRING THREAT to human health worldwide. From highly prevalent diseases such as HIV, dengue, and influenza to emerging viruses such as Ebola, SARS, and the Middle East respiratory syndrome coronavirus, viral infections cause serious diseases that require active treatment to prevent morbidity and mortality. Effective antiviral drugs are available for only nine viruses, including HIV, hepatitis B virus, hepatitis C virus, herpesvirus, influenza virus, human cytomegalovirus, varicella-zoster virus, respiratory syncytial virus, and external anogenital warts caused by human papillomavirus; however, most viral diseases lack effective drug treatments (De Clercq and Li, 2016).

The lack of narrow and broad spectrum antivirals is a deficiency in the modern medical formulary. This deficiency is due in large part to the genetic and replication-cycle diversity of viruses and the one-virus, one-drug mindset model used for current antiviral therapies. Most currently marketed antivirals have been developed to interact specifically with a viral protein from a virus of interest (e.g., HIV integrase or protease) and have not been shown to have significant utility against other viruses.

One approach to identifying broad-spectrum antivirals is to investigate host proteins or cofactors shown to be asso-

ciated with viral replication that can be targeted by small molecules in a way that blocks viral infection. Uncovering host proteins or metabolic pathways that are important for multiple viruses can open the door for developing broad spectrum inhibitors of viral infection.

It is widely recognized that viruses depend on host cellular proteins and machinery at multiple points in their replication cycles, making host factors that are broadly used by many viruses attractive antiviral targets. All viruses use the protein synthesis machinery to drive translation of their mRNA. Identifying components of the translation machinery that are used by multiple viral families offers a significant opportunity to develop a broad spectrum antiviral approach. Inhibiting eukaryotic initiation factor 5A (eIF5A) function has recently emerged as a potential target for this type of intervention.

eIF5A is a translation factor that undergoes a unique post-translational modification, called hypusination. Hypusination of eIF5A occurs at Lysine 50 through a two-enzyme cascade, where an aminobutyl moiety from the polyamine spermidine becomes incorporated (by deoxyhypusine synthase [DHPS]) and hydroxylated (by deoxyhypusine hydroxylase [DOHH]), forming the hypusine residue (Fig. 1).

FIG. 1. Cartoon representation of the hypusination pathway. eIF5A is hypusinated through a two enzyme cascade, where an aminobutyl group from the polyamine spermidine is first covalently attached to lysine 50 of eIF5A by deoxyhypusine synthase (DHPS) then hydroxylated by deoxyhypusine hydroxylase (DOHH). Small molecule inhibitors for each enzyme are also depicted.

FIG. 2. HIV requires eIF5A for Rev-dependent nucleocytoplasmic transport. **(A)** Schematic of Rev-dependent nucleocytoplasmic transport with (*left*) and without (*right*) eIF5A. Mutant-eIF5A inhibits nucleocytoplasmic transport. **(B)** Hypusination inhibitors also inhibit HIV replication. NPC, nuclear pore complex; RRE, Rev responsive element.

eIF5A is the only cellular protein that contains hypusine, and hypusination is critical for its eIF5A function (Park *et al.*, 1981). Hypusinated eIF5A (hyp-eIF5A) has been suggested to be involved in many cellular processes. Originally, hyp-eIF5A was identified as a stimulator of dipeptide synthesis (Kemper *et al.*, 1976; Schreier *et al.*, 1977; Benne and Hershey, 1978), but more recently has been suggested to be important for mRNA nucleocytoplasmic transport (Ruhl *et al.*, 1993; Bevec *et al.*, 1996; Elfgang *et al.*, 1999), mRNA stability (Zuk and Jacobson, 1998; Schrader *et al.*, 2006), and the translation of “hard to translate” regions, such as polyproline stretches (Gutierrez *et al.*, 2013). The cellular functions of eIF5A have been reviewed in detail elsewhere (Dever *et al.*, 2014; Rossi *et al.*, 2014).

Increasing evidence suggests that eIF5A can play an important role in modulating virus replication. HIV was the first virus suggested to require eIF5A, through dependence of Rev-dependent nucleocytoplasmic transport on eIF5A (Fig. 2). The HIV-1 Rev transactivator protein mediates the translocation of viral mRNAs from the nucleus to the cytoplasm, and as such is essential for the expression of viral structural proteins (Malim *et al.*, 1990). eIF5A specifically binds to Rev (Ruhl *et al.*, 1993) and eIF5A loss-of-function mutants blocked the nuclear export of Rev protein and HIV-1 replication (Bevec *et al.*, 1996). Furthermore, inhibition of the hypusination of eIF5A has also been shown to inhibit HIV replication (Andrus *et al.*, 1998; Hauber *et al.*, 2005; Hoque *et al.*, 2009; Schroeder *et al.*, 2014). Taken together, these data suggest that this pathway may be an interesting target for the development of HIV therapeutics.

Recent work by our group suggests that hyp-eIF5A also plays a role in the replication of the Filoviruses Ebola virus (EBOV) and Marburg virus (MARV) (Olsen *et al.*, 2016). Depletion of hyp-eIF5A followed by infection with EBOV or MARV resulted in a 3 log reduction of infectious titers of these viruses. Further mechanistic probing utilizing an EBOV transcription and replication minigenome assay revealed a defect in reporter gene expression, indicating that hyp-eIF5A is required for EBOV gene expression and/or genome replication. Furthermore, the accumulation of EBOV protein VP30 was reduced in the presence of hypusination inhibitors. Because VP30 is required for EBOV gene expression, a reduction in VP30 results in reduced reporter gene expression in the minigenome assay, and likely a halt in viral gene expression in viral infection (Fig. 3). These results suggest that targeting the hypusination of eIF5A may provide a therapeutic target for the development of antivirals treating hemorrhagic fever viruses.

The appreciation that multiple, diverse virus families require eIF5A for their replication suggests that blocking its function might be a broad spectrum antiviral approach. Several molecules have been developed to target the hypusination of eIF5A. The two-step hypusination pathway offers two enzymes that can be directly targeted. The compounds ciclopirox (CPX) and deferiprone (DEF), which are used clinically as topical antifungal agents, inhibit DOHH (Park *et al.*, 1996). In cell culture experiments, CPX and DEF reduce both HIV (Hoque *et al.*, 2009) and filovirus replication (Olsen *et al.*, 2016), making them strong candidates for drug repurposing. Furthermore, CPX has been

FIG. 3. Model representation of EBOV requirement of hypusinated-eIF5A for gene expression through a VP30-dependent mechanism. Hypusination inhibitors result in a reduction of VP30 mRNA translation, leading to a defect in EBOV secondary transcription. EBOV, Ebola virus; NP, nucleoprotein; VP30, viral protein 30; VP35, Viral protein 35; L, polymerase.

tested *in vivo* in animal models for various human diseases, and has recently been tested in a phase I clinical trial in patients with hematologic malignancies (reviewed by Shen and Huang, 2016). Another compound, CNI-1493 (semaphimod), is a guanylhydrazone that efficiently inhibits hypusination through targeting DHPS. CNI-1493 has also been shown to interfere with HIV-1 replication (Hauber *et al.*, 2005).

A second approach to inhibiting hypusination is to add a nonfunctional substrate that inhibits the hypusine synthesis enzymes. N1-guanyl-1,7-diamine-heptane (GC7) is a spermidine analogue and competitive inhibitor of DHS (Jakus *et al.*, 1993). Recent work shows that GC7 is an effective compound to control EBOV replication, suggesting that this compound may be an effective antiviral (Olsen *et al.*, 2016). Another spermidine analogue, 1,8 diaminooctane, has been shown to inhibit HIV replication *in vitro* (Hart *et al.*, 2002). These findings suggest that inhibition of hypusination through substrate competition at DHPS is possible. GC7 has been extensively studied as an antitumor agent (reviewed by Nakanishi and Cleveland, 2016), as well as *in vivo* for several applications (Imam *et al.*, 2014; Melis *et al.*, 2016), so animal model testing of GC7 efficacy is a logical next step in investigating the importance of eIF5A for virus replication and pathogenesis.

Virus replication can be attacked by blocking pathways upstream from eIF5A, through alteration of the levels of polyamines such as spermidine, spermine, and putrescine. Inhibition of polyamine synthesis pathways using compounds such as difluoromethylornithine (currently used to reduce trypanosome levels in infected individuals) results in a decrease in viral replication of numerous RNA viruses (Mounce *et al.*, 2016a, 2016b; Olsen *et al.*, 2016). Viruses sensitive to polyamine depletion include flaviviruses (dengue and Zika), bunyaviruses (Rift Valley fever), and picornaviruses (polio, Coxsackievirus) (Mounce *et al.*, 2016a). The exact mechanism for viral sensitivity to polyamine levels is not fully understood, but the requirement for spermidine in the hypusination of eIF5A could offer an explanation and will be important to study moving forward.

Potential Complications of Targeting eIF5A

Nonspecific side effects are always a concern associated when a cellular process is targeted as a way to block virus replication. There are certainly risks with targeting the host translational machinery generally as an antiviral strategy, as the normal synthesis of host proteins is an integral part of cellular and organismal homeostasis. Targeting the hypusination of eIF5A would likely have a more limited off-target effect than one would expect for global translation inhibition, as eIF5A is important for the translation of only a discreet subset of proteins (Fujimura *et al.*, 2015). The current compounds that block hypusination that are available to develop as antivirals may cause additional off-target effects. CPX targets multiple enzymes and pathways, including but not limited to ribonucleotide reductase, Wnt/ β -catenin, VEGF, VEGFR-3, mTOR, and cyclin-dependent kinases, which could lead to undesirable off-target effects as an antiviral, although it has been tolerated in animal studies (Shen and Huang, 2016). Similarly, CNI-1493 is a multivalent compound that interferes with several pathways, including the synthesis of TNF and the phosphorylation of p38 MAPK. Furthermore, the clinical applications of using a

spermidine analogue, such as 1,8 diaminooctane, are also unclear, given that it could potentially affect other enzymes responsive to spermidine. Toward alleviating some of these issues, novel compounds targeting DHPS, using the crystal structure of DHPS bound to GC7 as a design base, are being synthesized and tested for inhibition of HIV-1 replication (Schroeder *et al.*, 2014).

Targeting hypusination and eIF5A as a broad spectrum antiviral approach is an exciting possibility in early stages of development. Challenges remain for this idea in establishing whether this pathway can be an antiviral target, including *in vivo* testing in relevant animal models and the development of additional small molecule inhibitors/compounds that target the hypusination of eIF5A. Given the importance of hyp-eIF5A for viral replication and cancer biology, there is the opportunity for a multidisciplinary approach to address these concerns. Furthermore, through combining therapies targeting both host and viral targets, antiviral efficacy may improve, reducing the emergence of viral resistance, and minimizing toxicity in the control of viral infection and epidemic viral diseases.

Acknowledgments

We would like to thank Michaela Smith, Alexander Devaux, Emily Speranza, and John Ruedas for helpful comments on the article. This work was funded by NIH R21 AI121933 and NIH RO1 AI1096159-04 grants and by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number UC6A I058618.

Disclosure Statement

No competing financial interests exist.

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Received for publication December 19, 2016; accepted December 19, 2016.