Antiviral Research 99 (2013) 68–77

Contents lists available at SciVerse ScienceDirect

Antiviral Research

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

Review

Curing a viral infection by targeting the host: The example of cyclophilin inhibitors

Kai Lin [a,*], Philippe Gallay [b]

[a] Permeon Biologics, Inc., One Kendall Square, Cambridge, MA 02139, USA
[b] Department of Immunology & Microbial Science, The Scripps Research Institute, La Jolla, CA 92037, USA

ARTICLE INFO

Article history:
Received 25 January 2013
Revised 24 March 2013
Accepted 29 March 2013
Available online 8 April 2013

Keywords:
Host target
Antiviral
HCV
Cyclophilin

ABSTRACT

Every step of the viral life cycle is dependent on the host, which potentially can be explored for antiviral targets. Historically, however, drug discovery has focused mainly on viral targets, because of their perceived specificity. Efforts to pursue host targets have been largely hampered by concern over potential on-target toxicity, the lack of predictive cell culture and animal models, and the complexity of host–virus interactions. On the other hand, there are distinct advantages of targeting the host, such as creating a high barrier to resistance, providing broad coverage of different genotypes/serotypes and possibly even multiple viruses, and expanding the list of potential targets, when druggable viral targets are limited. Taking hepatitis C virus (HCV) as the example, there are more than 20 inhibitors of the viral protease, polymerase and NS5A protein currently in advanced clinical testing. However, resistance has become a main challenge with these direct-acting antivirals, because HCV, an RNA virus, is notoriously prone to mutation, and a single mutation in the viral target may prevent the binding of an inhibitor, and rendering it ineffective. Host cyclophilin inhibitors have shown promising effects both *in vitro* and in patients to prevent the emergence of resistance and to cure HCV infection, either alone or in combination with other agents. They are also capable of blocking the replication of a number of other viral pathogens. While the road to developing host-targeting antivirals has been less traveled, and significant challenges remain, delivering the most effective antiviral regimen, which may comprise inhibitors of both host and viral targets, should be well worth the effort.

Contents

1. Introduction

Viruses, particularly those with small genomes, encode only a few proteins of their own and depend on the host for many essential functions. Natural selection has therefore favored viruses that most efficiently hijack the host machinery. Hepatitis C virus (HCV) is a prime example. It enters cells by binding to the cell surface receptors CD81 (Pileri et al., 1998), human scavenger receptor class B type I (Scarselli et al., 2002), tight junction protein claudin-1 (Evans et al., 2007) and occludin (Ploss et al., 2009). Following decapsidation and release of its positive-strand RNA genome, the

* Corresponding author. Tel.: +1 781 999 4168.
E-mail address: kai.lin@ymail.com (K. Lin).

0166-3542/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2013.03.020

viral polyprotein is translated from the internal ribosomal entry site (IRES) at 5′-UTR by recruiting a number of cellular proteins including the eukaryotic initiation factors (Hellen and Pestova, 1999). The polyprotein is subsequently cleaved into individual viral proteins by the host signal peptidase and peptide peptidase, as well as viral proteases (Pène et al., 2009). A replication complex, formed by both viral nonstructural proteins and cellular co-factors on endoplasmic reticulum (ER)-derived membrane, synthesizes both negative- and positive-strand viral RNA (Romero-Brey et al., 2012). Virions containing the positive-strand viral RNA genome are assembled on ER-associated lipid droplets (LD) where the viral core protein is located, then bud through the ER lumen and Golgi to be released (Lindenbach 2013). In summary, essentially every step of the HCV life cycle is dependent on the host.

Given that host interactions are critical for viral replication, one would imagine that many host factors could be potential antiviral targets. However, antiviral drug discovery has historically been focused mainly on viral targets. Almost all currently approved antiviral drugs (excluding immunomodulators) and those under development target viral proteins. For example, all five licensed hepatitis B virus (HBV) inhibitors are nucleos(t)ide polymerase inhibitors (Fung et al., 2011). All herpesvirus drugs target the viral DNA polymerases (Price and Prichard, 2011). Currently there are only two classes of inhibitors for influenza, viral neuraminidase inhibitors and M2 channel blockers (Barik 2012). More than two dozen human immunodeficiency virus type-1 (HIV-1) drugs have been approved targeting the viral reverse transcriptase, protease or integrase (De Clercq 2010). The only host-targeting antiviral that has been successfully developed is maraviroc, a CCR5 inhibitor blocking HIV-1 entry (Dorr et al., 2005).

Hepatitis C has been the most active area for antiviral drug development over the past two decades, with two drugs already approved that inhibit the viral NS3 protease, and many more in development targeting the viral NS5B polymerase and NS5A protein. On the other hand, resistance has become a main challenge with these direct-acting antivirals, because of the high replication and mutation rates of the virus. An alternative and complementary strategy is to target host factors essential for viral replication, which may create a higher genetic barrier to resistance and could be used in combination with viral inhibitors. This review discusses opportunities and challenges in developing host-targeting antivirals, using cyclophilin inhibitors as a specific example.

2. Discovery of host antiviral targets

Both systemic screens and rational approaches have been employed to identify novel host factors that are essential for viral replication. Efforts have been taken to screen for cellular proteins that directly interact with specific proteins of HIV-1 (Jäger et al., 2011), influenza virus (Sharma et al., 2011), or HCV (Taguwa et al., 2008; Wang et al., 2006; Huang et al., 2013). However, methods such as yeast-two hybrid screening often result in hits that do not necessarily have functional relevance. Thus, it is important to confirm the finding through either loss (e.g. siRNA) or gain (e.g. ectopic expression) of function studies.

The most common approach is to screen a host siRNA library for targets that lead to an inhibition of viral replication when knocked down. Multiple screens have been performed for HIV-1 (Brass et al., 2008; Zhou et al., 2008), influenza virus (König et al., 2010), and HCV (Li et al., 2009; Tai et al., 2009). Interestingly, the percentage of overlapping hits from screens done by different groups on the same virus has been surprisingly low, which could result either from different assay conditions and/or a high rate of false positives. There were a few exceptions. For example, PI4Kα was reported as a hit in almost every HCV screen performed using either a subgenomic replicon or full-length infectious virus (Borawski et al., 2009; Tai et al., 2009; Berger et al., 2009; Trotard et al., 2009; Vaillancourt et al., 2009; Reiss et al., 2011). Such a reproducible hit suggests that the target is less likely to be affected by the conditions of the cells or assays, and may thus represent a more attractive candidate for drug discovery.

Whole cell-based phenotypic screens of large compound libraries have also generated a lot of leads, but with unknown targets or mechanisms (Kim et al., 2007; Chockalingam et al., 2010; Gastaminza et al., 2010). Compounds that inhibit viral targets can be easily ruled out by secondary screens; the remaining hits could then be candidates to identify potential host targets. The challenge is to deconvolute the pathway and identify the target, using chemogenetic approaches such as pull-down of the protein target using a labeled inhibitor. The advantage of such an approach is that, once the target is confirmed, the lead compounds have already been identified as potential chemistry starting points. Transcriptional and proteomic profiling comparing virus-infected cells to uninfected cells, or drug-treated vs. untreated cells, could also generate rich information on host–virus interactions (Su et al., 2002; Jacobs et al., 2005; Xu et al., 2012).

Sometimes targets are being pursued based on a specific hypothesis. Ribavirin was known to enhance the antiviral response when being used in combination with IFN-α. While the exact mechanism of ribavirin remains unclear, it is a weak inhibitor of inosine-5′-monophosphate dehydrogenase (IMPDH), which catalyzes the conversion of IMP to XMP, an essential step in the *de novo* biosynthesis of guanine nucleotides. Inhibition of IMPDH leads to a depletion of intracellular GTP pools and thus blocks viral replication. This hypothesis triggered the effort in developing a more potent and specific inhibitor of IMPDH, VX-497 (merimepodib), which indeed blocked HCV replication *in vitro* and showed some antiviral effect in patients (Markland et al., 2000; Marcellin et al., 2007).

A more focused approach is to analyze specific pathways that are known to be involved in viral replication. For example, it has been well characterized that HCV replicates on an ER-associated membrane web structure, and that HCV virions are assembled on ER-associated lipid droplets, both of which can be affected by host lipid biosynthesis (Romero-Brey et al., 2012; Lindenbach 2013). Thus, cellular proteins that are involved in lipid metabolism could be potential antiviral targets. Several studies have demonstrated that statins were able to inhibit HCV replication *in vitro* (Ikeda et al., 2006; Kim et al., 2007). A specific inhibitor of diglyceride acyltransferase-1 (DGAT-1) was reported to block HCV virion assembly and release (Herker et al., 2010). More recently, fatty acid synthase was proposed as another host antiviral target (Evanchik et al., 2012; Huang et al., 2013). Pathways involved in HCV replication, potential host targets and their known inhibitors are summarized in Table 1.

3. Myths and realities of host targets

While many host factors are known to be essential for viral replication, few have exploited for drug development because of the perceived hurdles (Table 2). The most common myth is that host targets are not specific, and thus may cause more toxicity. While inhibition of the normal cellular function of a host protein could potentially lead to target-related toxicities, there are many ways in which such a risk can be mitigated. First, a rapidly multiplying virus likely has a very different threshold for certain protein functions from those of normal cells, as reflected in the fact that many cellular pathways and genes are up-regulated in infected cells. It is therefore possible that knocking down these targets to their "normal" level may be sufficient to block viral replication, while main-