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REVIEW

Targeting host cofactors to inhibit viral infection

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Abstract **The majority of FDA-approved drugs indicated for the treatment of viral infections are inhibitors of viral proteins, of which the emergence of resistant strains is a major concern. This issue is exacerbated as most developed antiviral therapies are indicated for the treatment of viruses with error-prone replication. These problems may be addressed by the development of drugs that modulate the function of host factors involved in various aspects of a viral life cycle. Targeting host factors uncouples the mutation of a druggable protein gene from the replication and survival selection pressure exerted on a virus. Currently, a host-targeting antiviral (HTA), maraviroc, is approved for the treatment of human immunodeficiency virus (HIV) infection. In addition, several HTAs indicated for the treatment of hepatitis C virus (HCV) or HIV infection are at various stages of clinical evaluation. Targeting host factors is an attractive complement to therapies directly targeting a viral protein because of the expected higher genetic barrier for resistance and an overall increase in the diversity of treatment options. We examine how the integrated roles of emerging host cofactor screening approaches and drug development strategies may advance current treatment options.**

Keywords antiviral therapy, host-targeting, cofactors, drug resistance, HIV, HCV

Introduction

Viruses are obligate intracellular parasites that express relatively few classes of enzymes and lack their own metabolism. In contrast, host cells express thousands of genes from most enzyme classes. Viruses have become proficient at adapting their few proteins to the complex cellular milieu, in part by subverting host factors to sustain their own replication. However, efforts to develop antiviral compounds have thus far primarily focused on inhibiting viral proteins. For example, there are viral-targeted drugs currently on the market for many significant human pathogens including hepatitis C virus (HCV), HIV-1, and influenza A virus.

Viral enzymes with readily measurable functions (i.e. proteases) are especially amenable to drug development, as inhibitors of which can be screened outside a cell. The number of viral enzymes for a given virus, however, is typically very limited. A complementary strategy is to target host cofactors critical for viral replication but expendable to the cells, at least for the treatment period. While the precise set of host cofactors is not defined for any virus, it is likely that host targets are more numerous than viral targets. In addition, targeting host factors provides an important advantage owing to the relatively high barrier to drug resistance compared with direct viral targets, as evidenced by the universal emergence of resistant HIV-1 strains to monotherapies, the loss of efficacy of M2 and NA protein inhibitors for influenza A (Bright et al., 2006; Deyde et al., 2007; Reece, 2007), and the recent observation of protease inhibitor (PI)-resistant HCV strains (Sarrazin et al., 2007). Most of these resistant strains arise due to point mutations that reduce the affinity of an inhibitor for an enzyme (primary mutations) with other mutations compensating for any associated loss in fitness (secondary mutations). In many cases, the reliance on a host cofactor is complex and the loss of a critical cellular component cannot be remedied by point mutations. Furthermore, host genes are generally more conserved than viral genes, increasing the likelihood of obtaining an inhibitor with pan-genotypic or cross-clade activity. It is also possible that host proteins or pathways found important for a particular virus may already have been targeted by known drugs, albeit for a different indication. These drugs can work synergistically with viral-targeting or immunomodulatory agents. For example, the recent *in vivo*

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validation of a role for the cholesterol receptor NPC1L1 in HCV entry was facilitated by the availability of ezetimibe, an antagonist that has already received FDA approval for a nonviral indication (Sainz et al., 2012). In addition, members of the *Poxviridae* family were found to rely on Abl-family tyrosine kinases to complete their life cycle, and an Abl kinase inhibitor (Gleevec) indicated for the treatment of myelogenous leukemia demonstrated antiviral effect in mice (Reeves et al., 2005). Plausibly, at the systems biology level, all tolerable host inhibitors would narrow the range of host cofactors for subversion by viruses.

Despite these potential benefits, there are significant concerns with targeting cellular components. There is an inherent loss in specificity when a host instead of a viral target is inhibited, increasing the risk of off-target effects. Notably, many potential host targeting therapies are expected to exhibit significant cellular toxicity. In theory, these issues may be alleviated by gene function redundancy. In practice, host-targeting antivirals (HTA) are selected and chemically refined for low toxicity and high efficacy.

Identification of host cofactors for viral infection

Strategies to identify host cofactors are diverse. Affinity pull-down is perhaps the most direct approach for identifying cellular proteins that form stable complexes with viral proteins (Fig. 1A). The interaction of recombinant HCV envelope glycoprotein E2 with cluster of differentiation 81 (CD81) was discovered by affinity purification and was the first in a long line of evidence demonstrating a critical role of CD81 in HCV entry (Pileri et al., 1998). The yeast two-hybrid (Y2H) system is similar to affinity pull-downs in that it relies on direct protein interactions (Fig. 1B). The HCV cofactors VAP-A and -B were discovered by Y2H screening (Tu et al., 1999; Hamamoto et al., 2005). Arguably, an advantage of Y2H over an affinity pull-down is its amenability to high-throughput applications. Epstein-Barr virus was the first to receive the attention of an unbiased, genome-wide viral-host Y2H screen (Calderwood et al., 2007). Similar large-scale studies have since been carried out for other viruses including HCV (de Chassey et al., 2008) and influenza A (Shapira et al., 2009). Despite its wide applications, some intrinsic limitations of Y2H system persist; for example, false positive results can arise because of desegregated proteins or a higher concentration of proteins in the testing environment. Therefore, it is imperative to validate Y2H hits using complementary approaches and bioinformatics.

Humans and chimpanzees comprise the strict known host range of HCV infection. Efforts to establish a small animal model have brought attention to the molecular differences between murine (non-permissive) and human (permissive) hepatocytes. Approaches that make use of the difference between these cells to identify host factor rely on measuring gene expression levels (as in a microarray), or complementing non-permissive cells with functional gene expression (as in cDNA library screening). For example, the discovery that a liver-specific microRNA, miR-122, plays a critical role in HCV replication stemmed from the observation of a correlation between *in vitro* HCV permissiveness and miR-122 expression (Lagos-Quintana et al., 2002; Jopling et al., 2005). In addition, two of the HCV receptors, claudin (Evans et al., 2007) and occludin (Liu et al., 2009; Ploss et al., 2009), were identified using an iterative cDNA complementation. The number of false-positive hits in a microarray analysis can be significantly reduced by using cell lines that are highly similar to each other; and the functional complementation approach usually generates highly reliable results because of the gain-of-function design of the experiments.

Perhaps the most powerful of all these techniques is large-scale RNA interference (RNAi) screening (Fig. 1C). Other techniques are generally less sensitive in detecting low affinity interactions, transient or temporal events, signaling events, or interactions involving non-protein components. RNAi screening is applicable in all these areas and has the added benefit of detecting those factors deleterious to viral infection, known as host restriction factors. Still, RNAi screening is not without technical limitations, which include the inability to identify redundant factors or to completely suppress gene expression. Furthermore, the ability to perform an RNAi screen is limited by the availability of an amenable (i.e. transfectable, observable, and infectable) *in vitro* model, and the outcome of the screening project is influenced by the quality of the RNAi library. Notable genome-wide RNAi screens have been carried out for several viruses including HIV-1 (Brass et al., 2008; König et al., 2008; Zhou et al., 2008; Borner et al., 2010), HCV (Li et al., 2009), and influenza A (König et al., 2010). Among these, HIV-1 is the best studied, but a surprising lack of consensus has resulted from these investigations, each of which produced a long list of potential host cofactors (Bushman et al., 2009). Likely, differences in libraries, cell lines, and statistical methodologies will have to be considered to reduce the high number of false positives that currently plague RNAi screens. The immediate value of these lists of potential cofactors is the ability to guide subsequent experiments and compile information through meta-analyses.

The clinical relevance of cellular cofactors as drug targets becomes apparent when a chemical screening for antivirals generates compounds that target host proteins. Cyclosporine A (CsA), a known immunosuppressive drug, was such a compound for HCV. The discovery that CsA directly suppressed HCV infection *in vitro* (Watashi et al., 2003) led to the realization that cyclophilin A (CyPA), the main intracellular ligand of CsA, is an essential factor for HCV (Yang et al., 2008). These data have supported and facilitated clinical development of cyclophilin inhibitors (CPIs) as a new class of anti-HCV therapy. Conceivably, high throughput, cell-based compound screenings performed by large pharma-

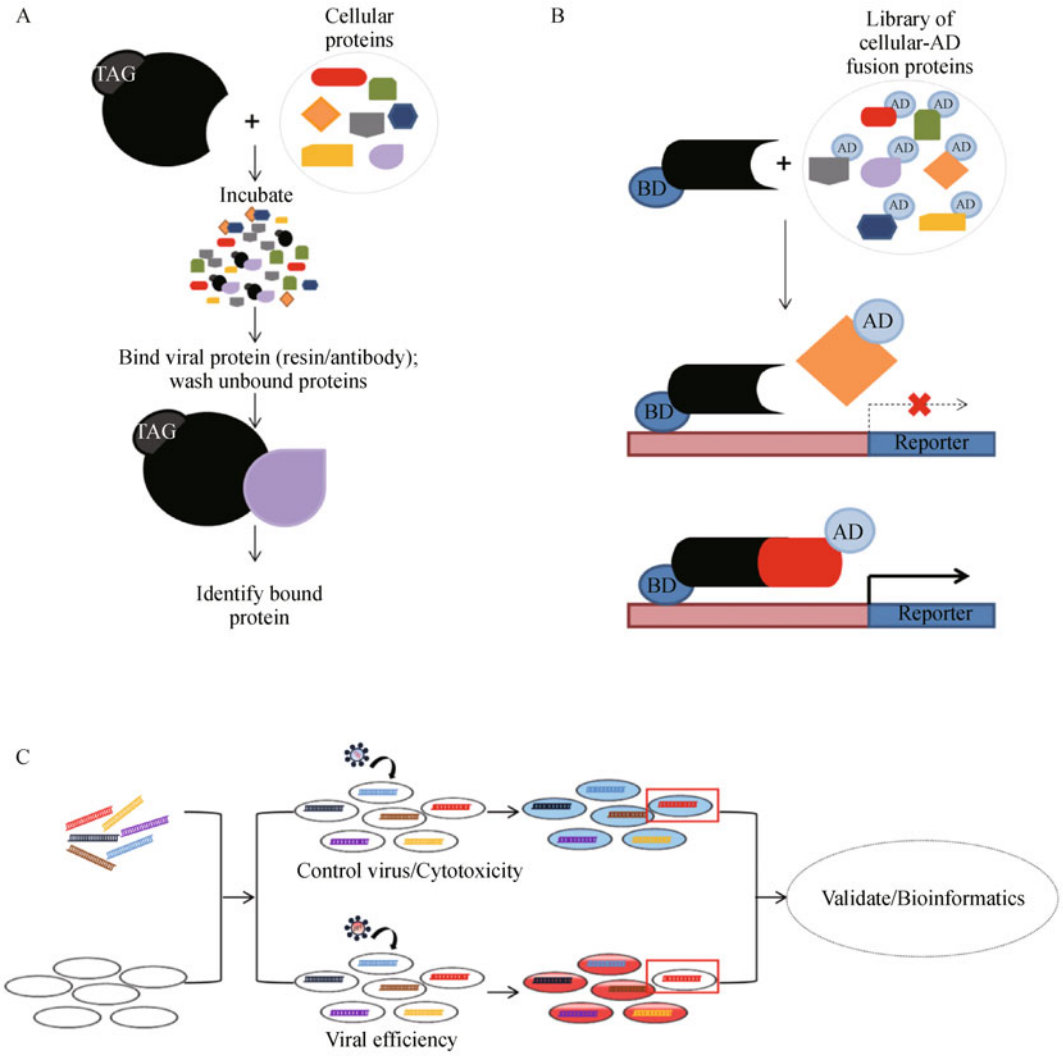

Figure 1 Schematic of major genome-wide cofactor identification methods. (A) Affinity pull-down. Tagged viral protein (black) is incubated with a sample of cellular proteins. The viral-host protein complex is then bound using resin or antibody. Unbound protein is washed away and bound cellular protein is identified. (B) Yeast two-hybrid system. Viral protein fused to a binding domain is challenged with a library of activating domain fusions. Successful matings are identified by colony formation or reporter assays. (C) RNAi screening. Cells are transfected (e.g. with siRNAs) or transduced (e.g. with shRNA vectors) by a selected library. Cells are then infected with the target virus and compared to a control virus and cytotoxicity screen.

ceutical companies, unless guided by target and structural information, should also produce host-targeting agents. Because these screens are often balanced by a counterscreen for specificity and cellular toxicity, the targets identified are likely to be non-essential for cell survival.

As the details of the host and virus interactions are made clearer, significantly more opportunities are likely to present themselves for drug development. Here, we examine two significant human pathogens for which host-targeting antivirals have demonstrated clinical efficiency: HCV and HIV-1. We will specifically exclude discussions of immunomodulatory drugs (e.g. interferons) or host environment-modifying drugs (e.g. matrix metalloprotease inhibitors). As these classes of drugs become more diverse it is likely that their respective subtleties will require examination by different lenses. One of the objectives of this review is to advocate a role for host-targeting antivirals (HTAs) in future drug development endeavors.

Hepatitis C virus

HCV is an enveloped, positive-sense RNA virus, infecting an estimated 3% of the world population. HCV infection leads to progressive liver disorders including fibrosis, cirrhosis, and hepatocellular carcinoma. As a result, it is one of the primary

worldwide indicators for liver transplantation. Its 9.6 kb genome is translated to a single polyprotein that is processed by host and viral proteases to generate the structural proteins core, E1, and E2, an ion channel protein p7, and the non-structural proteins NS2, NS3, NS4A/B, and NS5A/B.

The development of antiviral agents for the treatment of HCV was hampered by the lack of an infectious *in vitro* or small animal model. The first *in vitro* breakthrough was the development of the HCV replicon model in 1999 (Lohmann et al., 1999). Another significant advance occurred in 2005 with the discovery of a genotype 2a clone (JFH-1) that replicated efficiently in cell culture and released infectious particles, leading the establishment of a persistent model for the complete viral life cycle (Wakita et al., 2005). The availability of an infectious model in a transfectable hepatoma cell line has given HCV researchers a powerful and relevant system for drug development.

Until recently, the standard of care (SOC) for chronic HCV infection has been the combination of ribavirin and pegylated interferon (PEG-IFN), which is not effective in all patients. Sustained virological response (SVR), defined as a lack of detectable HCV RNA 24 weeks post-treatment, is achieved in approximately half of patients infected with genotype 1 HCV. Other patients fail to respond or relapse. In addition, this regimen is poorly tolerated with serious side effects. The mechanisms of treatment failure are not resolved, but it is clear that viral genotype, host genetics, comorbidities, and patient history are all contributing factors. The newly approved protease inhibitors significantly improved SVR rate but are still used in combination with the IFN regimen, and resistance to the protease inhibitors rapidly emerges *in vivo* (McHutchison et al., 2009; Kwo et al., 2010). As such, the development of continually more effective treatments, preferably IFN-free, is a major pharmaceutical initiative. Because HCV exhibits considerable diversity in its sequences among the different genotypes, and this diversity has a profound effect on the efficacy of the therapy, it is desirable to have a battery of treatment options, including HTAs, to combat this virus and potentially eradicate this "curable" infection.

Receptor targeting

HCV displays two viral glycoproteins on its envelope: E1 and E2. The role of E1 is undefined, but E2 has well-demonstrated affinity for binding various cell surface receptors. The precise mechanism of HCV entry is unclear, but it likely involves a multistep process engaging multiple attachment factors and viral receptors. Heparan sulfate proteoglycans (HSPGs) (Heo, 2008) and the low-density lipoprotein receptor (LDLr) (Nahmias et al., 2006) are the passive adsorption factors while CD81 (Pileri et al., 1998), scavenger receptor class B type I (SR-BI) (Scarselli et al., 2002), and the tight junction proteins claudin-1 (Evans et al., 2007) and occludin (Ploss et al., 2009) are actively involved in entry. In addition, the dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin (DC-SIGN), liver/lymph node-specific SIGN (L-SIGN) (Lozach et al., 2004), epidermal growth factor receptor (EGF-R), ephrin receptor A2 (EphA2) (Lupberger et al., 2011), asialoglycoprotein receptors (Saunier et al., 2003), and Niemann-Pick C1-like 1 cholesterol absorption receptor (NPC1L1) (Sainz et al., 2012) have all been reported to play a role in HCV entry.

CD81. The tetraspanin CD81 is a non-glycosylated protein containing four transmembrane and two extracellular domains. It binds to HCV E2 via its major extracellular loop. The important role of CD81 in HCV entry has been demonstrated by anti-CD81 antibodies and RNAi knockdown in various *in vitro* systems including primary human hepatocyte cultures infected by serum-derived HCV (Pileri et al., 1998; Flint et al., 1999; Bartosch et al., 2003; Zhang et al., 2004; Molina et al., 2008). In addition, Meuleman et al. reported that anti-CD81 monoclonal antibodies (mAbs) can effectively inhibit HCV infection in a human liver-uPA-SCID mouse model (Meuleman et al., 2008). Although no difference was observed between the anti-CD81 mAbs and an irrelevant antibody once the infection was established (i.e. 6 h after infection), these results nevertheless suggest a prophylactic effect by disruption of the E2/CD81 interaction and a potential in reducing the spread or severity of HCV infection with CD81 inhibitors, which are currently in preclinical development.

Scavenger Receptor BI. SR-BI is a 509 amino acid lipoprotein receptor with two transmembrane domains and is responsible for selective uptake of cholesteryl esters from high-density lipoproteins (HDLs) (Krieger, 2001). Compared to the ubiquitously expressed CD81 (Levy et al., 1998), its expression is limited largely to the liver and andrenal glands. This more restricted expression may lessen side effects exerted by targeting compounds. In addition, a mAb raised against SR-BI demonstrated antiviral effect *in vitro* and *in vivo* even when administered after infection, supporting a role of SR-BI in cell-to-cell transmission, in addition to cell-free infection (Lacek et al., 2012).

The most clinically advanced HCV entry inhibitor is the SR-BI antagonist ITX-5061 developed by iTherX, Inc. ITX-5061 is a small molecule first shown to increase HDL levels in hypertriglyceridemic patients. Because SR-BI is a selective receptor for HDL and because HDL levels failed to increase in SR-BI knockout mice treated with ITX-5061, ITX-5061 is likely a specific inhibitor for SR-BI (Masson et al., 2009). The discovery of SR-BI as an HCV entry receptor promoted the pursuit of this compound as a potential HCV therapy (Syder et al., 2011; Zhu et al., 2012). A phase 1b clinical trial of ITX-5061 with HCV-infected adults was recently concluded and evaluations are underway to determine the safety and efficacy of ITX-5061 in preventing the reestablishment of infection in liver transplant patients. *In vivo* resistance has yet to be evaluated, but a recent *in vitro* study showed high-level resistance to ITX-5061 conferred by mutations in E2 (Zhu et

al., 2012). One of these mutations, G451R, is known to increase the affinity of E2 for CD81 and reduces the EC_{50} of ITX-5061 by 50-fold (Zhong et al., 2006; Grove et al., 2008; Zhu et al., 2012). These results suggest that it will be necessary to target different entry receptors simultaneously for an entry inhibitor-based therapy to be successful.

Niemann-Pick C1-like 1 cholesterol absorption receptor. NPC1L1 is commonly found in the intestines of various species and known to be involved in overall cholesterol homeostasis. In humans and primates, it is also found on the apical surface of hepatocytes. NPC1L1 expression was recently demonstrated to be necessary for HCVcc entry by RNAi knockdown, mABs targeting NPC1L1, and a clinically licensed antagonist, ezetimibe (Sainz et al., 2012). Ezetimibe is currently on market for controlling cholesterol uptake. As cholesterol plays an important but undefined role in the HCV life cycle, the role of NPC1L1 in HCV entry could be indirect and mediated by cholesterol biogenesis. Still, ezetimibe may be a safe and ready addition to an effective combination therapy.

Therapies directed at components outside the cell to inhibit viral entry are complicated by the possibility of infection persisting through direct cell-to-cell transmission, which has been observed in other viruses including HIV (Jolly et al., 2004; Groot et al., 2008) and rhabdovirus (Iwasaki and Clark, 1975; Charlton and Casey, 1979). The contribution that cell-free and cell-to-cell transmission makes to HCV spreading in chronically infected patients is unknown (Timpe et al., 2008). *In vitro*, at least SR-BI, claudin-1, and occludin appear to influence cell-to-cell spreading efficiency (Brimacombe et al., 2011). Entry inhibitors may greatly compliment other therapies by reducing the overall genome count and inhibiting the spread of resistant genomes in infected patients.

Cyclophilin inhibitors

Cyclophilins (CyPs) are a family of proteins with peptidyl-prolyl isomerase activity and are high-affinity ligands of the cyclic nonribosomal peptide CsA, which is commonly used in organ transplantation as an immunosuppressant. CsA had been implicated in correcting liver pathology in HCV-infected patients as early as 1988 (Teraoka et al., 1988) although it did not receive much attention from the HCV field until a direct anti-viral effect was demonstrated in the replicon system 15 years later (Watashi et al., 2003).

CyPs were long suspected to play a role in CsA's anti-HCV effects, but it was not known which isoform if any was the primary regulator of HCV replication. Despite initial work indicating a genotype-dependent requirement of CyPB and/or CyPC (Watashi et al., 2005), more recent work demonstrated a broad and essential role of CyPA, but specifically not CyPB or CyPC, for HCV replication (Yang et al., 2008; Abe et al., 2009; Chatterji et al., 2009; Ciesek et al., 2009; Kaul et al., 2009).

FK506 (Tacrolimus) is another immunosuppressive agent and binds to FK506 binding proteins (FKBPs). Both the CsA/CyPA and FK506/FKBP complexes bind calcineurin and block its phosphatase activity, which is critical for T cell activation (Liu et al., 1991). However, FK506 does not inhibit HCV replication. Furthermore, CsA binding inhibits CyPA's isomerase activity, which is dispensable for calcineurin inhibition (Zydowsky et al., 1992), but critical for HCV replication (Chatterji et al., 2009; Liu et al., 2009; Foster et al., 2011). These results suggest CsA's role in the HCV life cycle is independent of its immunosuppressive property. Indeed, several non-immunosuppressive derivatives of CsA that retain their anti-HCV function have been developed (Tang, 2010). These derivatives are modified at critical positions required for the formation of the CsA/CyPA/calcineurin quaternary structure (Liu et al., 1991; Landrieu et al., 2010; Tang, 2010) and represent more attractive candidates for HCV treatment. In addition, a class of naturally occurring, non-CsA-derivative cyclophilin inhibitors, the sanglifehrins, have recently been shown to have anti-HCV effect as well (Gregory et al., 2011).

Several CPIs have undergone clinical evaluation for the treatment of HCV infection: alisporivir (DEBIO-025), NIM811, and SCY-635. The most clinically advanced, alisporivir, was developed by Debiopharm and in-licensed by Novartis Pharmaceuticals. It has been shown to be highly effective at inhibiting HCV replication in cell culture (Paeshuyse et al., 2006), mice (Inoue et al., 2007), and humans (Flisiak et al., 2008, 2009). It was first evaluated in a small group of HIV/HCV coinfected patients and exhibited a specific antiviral effect against HCV (Flisiak et al., 2008). Dosage trials with alisporivir and IFN indicated a strong synergistic effect between the two drugs and a high cure rate (Flisiak et al., 2009). An international trial including ribavirin also achieved promising SVR rates, and a significant difference in relapse between alisporivir monotherapy (18%) and alisporivir with ribavirin (4%–9%) was observed, supporting a consistent but poorly-defined contribution of ribavirin in controlling relapse. This study was especially notable for excluding IFN treatment (and hence the side effects associated with it) while retaining high treatment indices (Pawlotsky, 2012). Even though several phase three trials had originally been scheduled to begin or continue in 2012, clinical evaluation of alisporivir has currently been put on hold due to concerns over pancreatitis. It remains to be seen if further safety testing will be carried out.

SCY-635 is under development by Scynexis, Inc. A phase 1b clinical study of patients infected with genotype 1 HCV receiving up to 900 mg SCY-635 demonstrated the promise of SCY635 monotherapy in reducing HCV serum RNA with no serious adverse effects (Hopkins et al., 2009). SCY-635 has also been suggested to reactivate innate IFN response which, in addition to HCV RNA reduction, correlated with a favorable IL28B genotype (Hopkins et al., 2012). A phase 2a trial is currently ongoing to test the effectiveness of a 28-day triple therapy with SCY-635 and IFN/RBV.

NIM811, a CsA derivative originally developed for HIV treatment, has also been evaluated for HCV inhibition *in vitro* and *In vivo*. In the replicon system, it suppressed HCV replication additively with PIs and NS5B inhibitors. Importantly, the emergence of resistance mutations were delayed by combination treatments and no single mutation that could confer cross-resistance to multiple drugs was identified, supporting a role of CPIs in a combination therapy (Mathy et al., 2008). Phase 1 study in healthy volunteers suggested a good safety profile. Based on the enhanced effect of NIM811 over CsA in the replicon system and the reported clinical efficacy of CsA, investigators in a phase 2 study projected antiviral effect with 400 mg of NIM811. However, NIM811 monotherapy was ineffective in the highest dose tested (600 mg), in contrast to alisporivir and SCY635 (Lawitz et al., 2011). Nevertheless, when added to an interferon arm, NIM811 was effective. It will be interesting to determine the molecular basis of the different effects of distinct CPIs in monotherapy.

MicroRNA-122

MicroRNAs (miRNAs) are short, non-coding RNA molecules known to function primarily in the post-transcriptional repression of gene expression. The expression of miRNA themselves can be tissue-specific and plays a broad role in diverse biologic processes including viral infection.

A liver-specific human miRNA, miR-122, was identified to be a critical cofactor for HCV infection in cell culture (Jopling et al., 2005). Because miRNAs generally bind to the 3′ UTR of a target mRNA to suppress gene expression, it was surprising when miR-122 was found to play a supporting role in the HCV life cycle. It interacts with two conserved positions in the 5′ UTR of HCV genome and may lead to RNA stabilization or translation enhancement (Jopling et al., 2005, 2008; Henke et al., 2008; Shimakami et al., 2012a, 2012b).

A pharmaceutical approach to miRNA inhibition is through the administration of locked nucleic acids (LNAs). LNAs are RNA nucleotides in which the ribose sugar is locked by an oxymethylene bridge connecting the 2′ and 4′ residues. This unique modification restricts the RNA conformation to the N-type sugar pucker (Koshkin et al., 1998; Obika et al., 1998; Singh et al., 1998), which improves Watson-Crick base-pairing selectivity and enhances stability in cells, contributing to a favorable therapeutic index (Vester and Wengel, 2004; Jepsen et al., 2004; Veedu and Wengel, 2010).

A miR-122-complementary LNA developed by Santaris Pharma, miravirsen (SPC3649- MIR), was injected intravenously in HCV-infected chimpanzees and displayed significant antiviral potential at high dose with no evidence of viral breakthrough or side effects (Lanford et al., 2010). In uninfected cynomolgus monkeys this treatment was also well tolerated (Hildebrandt-Eriksen et al., 2012). A phase 2a study of MIR suggested an infrequent treatment of once per month may be sufficient to maintain effective dosage, a promising factor in treatment adherence of an injection drug (Reesink et al., 2012). Four of nine patients treated with high-dose MIR monotherapy had undetectable HCV in their sera at the end of the 18 week trial.

MIR is unique and exciting as its clinical trials have demonstrated a proof-of-concept for a miRNA-targeting antiviral therapy, in addition to further validating antisense technology. Several lines of evidence indicate that long-term suppression of miR-122 may not be advisable. First, the entire mature miR-122 sequence, not just the seed region, is highly conserved in all species that express this microRNA. The lack of paralogues across species is distinct from many other microRNAs and hints at a conserved cellular function mediated by sequence outside the seed region. Secondly, the extremely high endogenous expression level of miR-122 in the liver (72% of total microRNAs) (Chang et al., 2004) and the evidence of miR-122 as a tumor suppressor (Kutay et al., 2006; Bai et al., 2009; Coulouarn et al., 2009) suggest the importance of maintaining miR-122 expression in normal liver tissue. Fortunately, the relatively short time frame needed to suppress HCV in chimpanzees was not associated with apparent liver toxicity (Lanford et al., 2010), making miR-122 one of the most promising host targets for HCV drug development.

Human immunodeficiency virus

HIV is a lentivirus and the causative agent of acquired immunodeficiency syndrome (AIDS). In 2010, the World Health Organization estimated that 34 million people worldwide were living with HIV with 1.8 million deaths that year attributable to AIDS (WHO/UNAIDS/UNICEF, 2011).

Despite a highly successful strategy involving multiple synergistic drugs to treat HIV, termed highly active antiretroviral therapy (HAART), HIV infection is generally regarded as incurable because of the integration of its genetic material into the host genome. In addition, the highly error-prone nature of the viral replicase (reverse transcriptase) and HIV's high turnover rate result in substantial genetic variation, leading to viral resistance (Roberts et al. 1988; Preston et al., 1988; Coffin, 1995; Ho et al., 1995). The life-long persistence of infection provides even greater opportunities for the development of drug resistance, making additional novel treatments with high genetic barriers desirable.

Currently there are more than two dozen FDA approved drugs for the treatment of HIV infection (Wilkin and Gulick, 2012). Drugs designed to treat HIV are directed at controlling the spread of the virus, reducing viral load and turnover, and maintaining the individual's immune system. While the majority of these drugs are direct inhibitors of HIV proteins, we now detail some of the significant advances in host-targeting HIV therapies.

Targeting binding and entry host cofactors

CD4. CD4 was identified as the primary receptor mediating HIV entry shortly after the virus was determined to be the causative agent of AIDS (Klatzmann et al., 1984; Dalgleish et al., 1984; McDougal et al., 1986). Recombinant, soluble CD4 is a high affinity binding partner of the viral glycoprotein gp120 and can block viral infection *in vitro*, originally suggesting a role of CD4 blockade in HIV therapy (Smith et al., 1987; Deen et al., 1988). TMB-355 (ibalizumab) is a non-immunosuppressive, anti-CD4 monoclonal antibody under development by TaiMed Biologics (Bruno and Jacobson, 2010). It recognizes the interface between domains 1 and 2 of CD4, decreasing the flexibility of the receptor and preventing bound gp120 from accessing the necessary coreceptor that mediates viral entry into target cells (Song et al., 2010). Ibalizumab is currently in an extended phase 2 clinical trial in addition to a safety trial on its prophylactic value in high-risk, healthy volunteers.

CCR5. While CD4 is the primary receptor for HIV entry, it is not sufficient for viral fusion with the target cell membrane. CXCR4 and CCR5 serve as the major coreceptors for the entry of HIV-1, of the X4 and R5 viruses, respectively (Alkhatib et al., 1996; Berson et al., 1996; Deng et al., 1996; Dragic et al., 1996; Feng et al., 1996). Binding of HIV-1 gp120 to the target cell CD4 induces a conformational change in the envelope protein that allows interaction with the coreceptor, and subsequent additional conformational changes in the Env lead to insertion of gp41 fusion peptides into the host membrane and gp41-mediated fusion (Chan and Kim, 1998; Kwong et al., 1998). CCR5-utilizing HIV-1 strains (R5 tropic) account for the majority of new infections and majority of current infections. Individuals homozygous for the 32-bp deletion in CCR5 (CCR5Δ32) failed to display CCR5 on their cell surface and demonstrate high levels of resistance to HIV-1 infection (Liu et al., 1996; Samson et al., 1996). While these individuals lack a functional CCR5 protein, they are generally regarded as healthy and immuno-competent. Thus, this natural genetic variant implicates CCR5 as a viable target for anti-viral treatment.

Several CCR5 antagonists have been investigated for therapeutic use. The allosteric CCR5 modulator maraviroc (Pfizer) is the only FDA-approved CCR5 antagonist to date, and potently inhibits R5-tropic virus *in vivo* (Kuritzkes et al., 2008). Cenicriviroc, an orally available CCR5 antagonist being developed by Tobira Therapeutics, is currently in phase 2b clinical trials (Klibanov et al., 2010). Clinical development of two more CCR5 antagonists has been suspended due to poor synergy with combination treatment (in the case of vicriviroc) or toxicity (in the case of aplaviroc) (Nichols et al., 2008; Wilkin and Gulick, 2012). Resistance to maraviroc and other CCR5 inhibitors has been identified both *in vitro* and *in vivo*. The resistance appears to be the result of either outgrowth of preexisting X4 virus pools in a patient (Westby et al., 2006) or from mutations, commonly in the V3 loop of gp120. Interestingly, these mutations allow for utilization of the drug-bound coreceptor instead of producing an R5 to X4 tropism switching (Trkola et al., 2002; Tilton et al., 2010; Roche et al., 2011). Accordingly, tropism-switching may present a higher genetic barrier as genetic intermediates can have reduced replication fitness and be more susceptible to inhibition (Pastore et al., 2004).

In light of the ability of some resistant R5-tropic strains to employ the drug-bound conformation of CCR5, and the fact that pre-existing CCR5 antagonist-resistant R5 variants may be present in a host prior to treatment, additional methods of making CCR5 inaccessible for viral use are needed. Development of CCR5 agonists that induce receptor internalization and inhibit its recycling could provide an additional barrier to drug resistance or viral escape mutants in combination therapies. The natural CCR5 ligand RANTES competitively blocks R5 tropic HIV-1 entry through agonist-induced internalization (Cocchi et al., 1995; Amara et al., 1997; Mack et al., 1998). However, its chemotactic nature precludes it as a potential treatment option. Non-signaling RANTES-derivatives are currently under investigation for use as anti-HIV agents. AOP-RANTES was the first of several synthetically modified RANTES reported to have anti-HIV activity (Simmons et al., 1997). PSC-RANTES, another chemically modified RANTES analog, was found to be 50 times more potent in inhibiting HIV entry than AOP-RANTES (Hartley et al., 2004), and is a highly effective microbicide in macaques (Lederman et al., 2004). More recently, Gaertner et al. reported several fully recombinant N-terminally modified variants of PSC-RANTES that are potent inhibitors of HIV infection that could provide a cheaper alternative to chemically modified chemokines (Gaertner et al., 2008). Zhao et al. further enhanced the anti-viral properties of two of these compounds, 5P12-RANTES, which does not trigger receptor internalization, and 5P14-RANTES, which actively sequesters CCR5, by linking the RANTES analogs to a known gp41-fusion inhibitor (Zhao et al., 2011). In addition, resistance to 5P12-RANTES is dependent upon a complete tropism switch, providing a higher barrier to resistance (Nedellec et al., 2011).

Monoclonal antibodies against CCR5 have also been pursued as potential therapeutic option. The monoclonal antibody PRO140 (Progenics Pharmaceuticals) exhibited potent antiviral activity for two to three weeks after a single, subcutaneous, infusion in a phase 2a clinical trial (Jacobson et al., 2010). Currently, a phase 2b trial is underway targeting viral rebound patients and those with documented poor adherence (Arnettet et al., 2011).

CXCR4. CXCR4 is the principal coreceptor for X4-tropic HIV-1 strains. Its natural ligand, stromal cell-derived factor 1 (SDF-1), inhibits infection of T cells by X4 HIV-1 (Bleul et al., 1996; Oberlin et al., 1996). In contrast to CCR5, no homozygous mutations of CXCR4 are known, and knockout of either CXCR4 or SDF-1 in mice results in hematopoetic and cardiac defects, implicating a broad role of CXCR4 in

development (Nagasawa et al., 1996; Zou et al., 1998). Because of the obvious concerns regarding toxicity based on these results, the development of a CXCR4 antagonist effective for safe treatment of HIV-1-positive patients has lagged behind that of CCR5-targeting drugs.

Inclusion of a CXCR4-targeting component, however, may be required for the optimization of a regimen aimed at HIV entry. Dual R5/X4 variants and mixed populations (R5, X4, and dual) occur in 20%–50% of individuals (Brumme et al., 2005; Moyle et al., 2005; Wilkin et al., 2007). Patients harboring X4 virus generally have a lower $CD4^+$ T cell count and a quicker clinical progression to AIDS (Richman and Bozzette, 1994). Higher prevalence of X4 tropism is correlated with later stage infections and previous exposure to antiretroviral therapy. These data suggest a role for CXCR4 inhibitors in combination therapies with maraviroc or other CCR5 antagonists.

While CXCR4-targeting compounds have shown potent antiviral activity *in vitro*, none so far have passed safety tests in clinical trials. The bicyclam AMD3100 was one of the earliest CXCR4 antagonists to go to clinical trials. Whereas it inhibited HIV-1 infection at the nanomolar range in cell culture (Donzella et al., 1998), clinical trials proved the compound to be ineffective in reducing overall HIV viral load in dual/mixed (D/M) or R5-tropic populations. Significant leukocytosis or cardiotoxicity and lack of oral bioavailability were also factors in the failure of AMD3100 (Hendrix et al., 2004). The X4 Antagonist Concept Trial in 2008 was a short study investigating the efficacy of the orally bioavailable non-cyclam AMD11070 (AMD070) against D/M virus. A more potent reduction of X4 viral load was observed, but hepatotoxic effects seen in long-term animal studies halted the development of AMD070, with additional studies needed to confirm safety for long-term use in humans (Moyle et al., 2009).

Entry Inhibitors as pre-exposure prophylaxis. Lacking an effective vaccine, targeting host factors involved in viral entry to prevent initial infection by HIV in high risk individuals could eliminate a large percentage of new infections. Recently, Truvada (Gilead Sciences), a pill which combines the two antivirals tenofovir and emtribitabine, was shown to have strong preventative effects in high risk populations, specifically men who have sex with men (msm) (Grant et al., 2010). The previously mentioned CCR5 antagonist maraviroc is also being investigated for its potential use in preventative treatment. Orally delivered maraviroc was found to be particularly concentrated in cervical-vaginal fluid and vaginal tissue, suggesting its usefulness as an HIV prophylactic in high-risk women (Dumond et al., 2009). Vaginally delivered maraviroc provided high levels of protection against CCR5-utilizing HIV-1 in a macaque model study (Veazey et al., 2010). RANTES analogs have also shown promise for use as microbicides in preclinical studies. High concentrations of vaginally-delivered PSC-RANTES were shown to fully protect macaques from challenge with R5-tropic HIV-1 (Lederman et al., 2004). The cyclotriazadisulfonamide CADA has been reported to inhibit gp120-CD4 binding through downregulating CD4 expression and, if safe *in vivo*, may be a potential microbicide (Vermeire and Schols, 2005; Vermeire et al., 2008). Effective microbicides targeting host factors could provide high risk populations with potential cross-clade topical protection.

LEDGF/p75

The lens epithelium-derived growth factor (LEDGF/p75), a chromatin binding cellular protein, has been shown to be involved in HIV replication through tethering the preintegration complex (PIC) onto host chromatin (Cherepanov et al., 2003; Maertens et al., 2003; Emiliani et al., 2005). A series of small molecule compounds (2-(quinolin-3-yl)acetic acid derivatives) known as LEDGINs that bind to the LEDGF/p75 binding pocket of HIV integrase (IN), but not to the host factor itself, have proven effective in preventing HIV integration *in vitro* (Christ et al., 2010). In addition, small molecules that target the same interaction but bind to the cellular partner LEDGF/p75 have also been reported (Cavalluzzo et al., 2012). These molecules (CAB) mimic the alpha-3 helix of IN and bind to the integrase binding domain (IBD) of LEDGF/p75. This binding inhibits protein–protein interaction between LEDGF/p75 and the full-length IN. Whether compounds aimed at disrupting the LEDGF/p75-IN interaction, either the allosteric IN inhibitors (LEDGINs) or the HTA compounds CABs, can progress into the clinical stage of HIV drug development remains to be seen.

Summary

The outbreak of the global HIV crisis prompted many important advances in the treatment strategies for viral infections. Due to HIV's incurability, necessity for lifelong treatment, and high mortality rate in untreated individuals, intense research efforts were directed at developing synergistic drugs with distinct resistance profiles. However, while treatment outlooks continue to improve, drug resistance has reduced the clinical value of all pathogen-targeting therapies, albeit to various degrees. Although simply combining more drugs can effectively reduce the impact of drug resistance, a more direct response is to target those factors which are not selected by the survival pressure of the pathogen. This emerging strategy is directly supported by advances in bioinformatics analyses and genome-wide screening technologies. For instance, genome-wide screens can highlight a focused list of drug targets from a much larger pool.

A comprehensive strategy for treating an infection should address the entire infectious, disease-causing unit, which includes the machineries of the host hijacked by the pathogen. With the success of the CCR5 antagonist maraviroc in the

clinic and the promising lines of new research, including the ones summarized in this review, we are confident that more HTAs will be an integral part of future anti-infective therapies.

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