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TOPIC HIGHLIGHT

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How hepatitis C virus invades hepatocytes: The mystery of viral entry

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Abstract

Hepatitis C virus (HCV) infection is a global health problem, with an estimated 170 million people being chronically infected. HCV cell entry is a complex multi-step process, involving several cellular factors that trigger virus uptake into the hepatocytes. The high- density lipoprotein receptor scavenger receptor class B type I, tetraspanin CD81, tight junction protein claudin-1, and occludin are the main receptors that mediate the initial step of HCV infection. In addition, the virus uses cell receptor tyrosine kinases as entry regulators, such as epidermal growth factor receptor and ephrin receptor A2. This review summarizes the current understanding about how cell surface molecules are involved in HCV attachment, internalization, and membrane fusion, and how host cell kinases regulate virus entry. The advances of the potential antiviral agents targeting this process are introduced.

Key words: Hepatitis C virus; Virus entry; Hepatocytes; Receptor; Host kinase; Antiviral target

Core tip: Cell entry is the first step in viral infection and replication, which offers an important target for antiviral therapy. Hepatitis C virus (HCV) cell entry is a complex multi-step process, involving a several cellular factors that trigger virus uptake into the hepatocytes. This review summarizes the current understanding about how cell surface molecules are involved in HCV attachment, internalization, and membrane fusion, and how host cell kinases regulate virus entry. The advances of the potential antiviral agents targeting this process are introduced.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem. About 130-170 million people are chronically infected and at risk of developing liver cirrhosis and/or liver cancer[1]. More than 350000 people die from hepatitis-C-related liver diseases every year. Current antiviral treatment of chronic HCV infection is based on pegylated interferon (PEG-IFN) and ribavirin, which is limited by side effects and suboptimal response rates[2]. Since 2011, standard of care (SOC) treatment of HCV includes the addition of direct-acting antivirals with a protease inhibitor to the PEG-IFN and ribavirin[3,4]. However, resistance and severe side effects are still issues[5]. There is currently no vaccine for hepatitis C. Thus, novel preventive and therapeutic strategies are urgently needed.

Cell entry is the first step in viral infection and replica-

tion, which offers an important target for antiviral therapy. The establishment of infectious HCV pseudoparticles (HCVpps)[6,7], cell-culture-derived HCV (HCVcc)[8-10], and progress in small animal models of HCV infection[11-14] have allowed us to identify host factors contributing to HCV entry and to develop antiviral compounds.

HCV entry into hepatocytes is thought to be a highly coordinated process that involves several cell surface molecules in sequential steps[15]. More than two decades of intense research has provided a detailed understanding of these entry factors, and considerable progress has been made to decipher how they trigger and facilitate HCV cell entry. Recent data also demonstrate the important role of host cell tyrosine protein kinases in regulating HCV entry[16]. In this review, we summarize the current knowledge about how HCV uses these host factors to invade hepatocytes, and how host cell kinases regulate virus entry, and the recent progress of antiviral therapy targeting this process.

HCV PARTICLES

HCV is an enveloped, positive-stranded RNA virus belonging to the Hepacivirus in the Flaviviridae family. The HCV virion is comprised of a nucleocapsid surrounded by a host-derived membrane containing the E1 and E2 HCV glycoproteins[17]. Functional virion-associated E1 and E2 are thought to form a non-covalent heterodimer stabilized by disulfide bridges[18,19], which mediate the majority of cell-entry processes of the virion, including interaction with host receptors and fusion in the low pH environment of early endosomes[20-23]. The first 27 amino acids residues of the N terminus of E2, called hypervariable region 1 (HVR1), are the most divergent among HCV isolates. Recent data show that HVR1 contains three different functional microdomains that cooperate to confer HCV cell entry and immune evasion[24]. Based on similarities with envelope proteins of the Flaviviridae family, E2 was previously proposed to be the fusion protein[25-27]. However, recent data on pestiviruses and HCV are no longer in favor of E2 being a fusion protein. The structure of E2 protein from the pestivirus bovine viral diarrhea virus does not show any evidence of a fusion peptide in this protein[28,29]. More recently, the crystal structure of the E2 core bound to broadly neutralizing antibody AR3C was resolved and revealed a compact architecture composed of a central immunoglobulin-fold β sandwich flanked by two additional protein layers, which differs markedly from predictions of an extended, three-domain, class Ⅱ fusion protein fold[25]. E1 was recently demonstrated to be a modulator of HCV binding to receptors and membrane fusion[30]. The characterization of HCV particles circulating in patient sera is the heterogeneity of size and density, due to the association of virions with different serum components, such as immunoglobulins[31] and different classes of lipoproteins[32].The nature of the association between HCV and lipoproteins remains unclear. Circulating virions might directly bind to low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in the blood, or interact with lipoprotein components during virus assembly and are secreted together with VLDL[33]. Highly infectious HCV particles are associated with VLDL, termed lipoviral particles (LVPs). LVPs are lipoprotein-like structures composed of triglyceride-rich lipoproteins containing apolipoprotein (Apo)B, ApoE and ApoC1, viral nucleocapsids, and envelope glycoproteins E1 and E2[34-38]. Several functional roles for the formation of HCV-lipoprotein complexes have been proposed, including interacting with lipoprotein receptors for attachment and entry, concealing epitopes to facilitate immune escape, and hijacking host factors for HCV maturation and secretion[39].

PARTICIPANTS AND THE CELL-ENTRY PATHWAY

The HCV entry process into human hepatocytes requires numerous host proteins, including two binding factors glycosaminoglycans (GAGs)[40,41] and the LDL receptor (LDLR)[42-45], four receptors scavenger receptor class B type I (SR-BI)[46], tetraspanin CD81[47], tight junction proteins, claudin-1 (CLDN1)[48] and occludin (OCLN)[49], and several entry factors including epidermal growth factor receptor (EGFR), ephrin receptor A2 (EphA2)[50], transferrin receptor 1 (TfR1)[51], and cholesterol transporter Niemann-Pick C1-like 1 (NPC1L1)[52] (Table 1). The entry pathway consists of three steps: (1) viral attachment to the hepatocyte; (2) receptor-mediated endocytosis of the viral particle; and (3) endosomal fusion (Figure 1).

ATTACHMENT OF HCV PARTICLES TO THE CELL SURFACE

In vivo, circulating HCV enters the liver through the sinusoidal blood. The liver sinusoidal endothelial cells (LSECs) and Kupffer cells express liver/lymph-node-specific intercellular adhesion molecule 3-grabbing integrin (L-SIGN) and dendritic-cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), respectively. Both L-SIGN and DC-SIGN bind HCV E2 with high affinity, suggesting they may capture particles from blood and transmit virus to hepatocytes[53,54].

Successful traverse from the endothelium allows the virus to contact hepatocytes at the basal surface. GAGs and LDLR are proposed to contribute to concentrate HCV particles on target cell surface[41,44]. On hepatocytes, highly sulfated GAGs (HS GAGs) serve as the first attachment sites[55]. While direct E2 binding to HS GAGs is observed[55], E1 and ApoE are also suggested to attach to GAGs[56].

As a result of the association between HCV and lipoproteins, the LDLR has been proposed as another potential attachment factor for HCV[43]. Although HCV particles bind to the LDLR, it seems that this interaction does not lead to a productive entry process[57].

Table 1 Characterization and the main function of cell surface molecules required for hepatitis C virus entry

	Characterization	Main function in HCV entry
GAGs	Binding factor	Attachment to HCV E1 and ApoE
LDL-R	Binding factor	Binding to virion-associated lipoprotein
SR-BI	Receptor	Binding to virion-associated lipoprotein; lipid transfer; binding to E2 HVR1
CD81	Receptor	Interaction with E2; signaling pathway activation which promotes actin remodeling
CLDN1	Receptor	Interaction with HCV-CD81 complex
OCLN	Receptor	Function at the late stage of HCV entry
EGFR and EphA2	Entry factor	Signaling pathways activation which lead to the formation of CD81-CLDN1 complexes
TfR1	Entry factor	Possibly involved in virus internalization
NPC1L1	Entry factor	Possibly involved in endosomal fusion

HCV: Hepatitis C virus; GAGs: Glycosaminoglycans; LDL: Low-density lipoprotein; CLDN1: Claudin-1; OCLN: Occludin; EGFR: Epidermal growth factor receptor; EphA2: Ephrin receptor A2; TfR1: Transferrin receptor 1; NPC1L1: Niemann-Pick C1-like 1.

ENDOCYTOSIS MEDIATED BY CO-RECEPTORS AND SPECIFIC ENTRY FACTORS

After initial attachment to the host cell surface, the virus interacts with co-receptors and other specific entry factors, which leads to molecular rearrangements at the plasma membrane and subsequently results in viral internalization.

SR-BI is highly expressed in the liver and steroidogenic tissues[58], can bind a variety of lipoproteins including high-density lipoprotein (HDL), LDL, and oxidized LDL[59], and plays a key role in mediating selective cholesterol uptake from HDL into hepatocytes to maintain lipid homeostasis[60]. SR-BI is a glycoprotein with two N- and C-terminal cytoplasmic domains separated by a large extracellular domain that is involved in lipid metabolism. The large extracellular loop of SR-BI was initially identified to bind directly to the HVR1 of HCV E2[61], and then interact with virus-associated lipoproteins[62]. Recent data show that HCV particles utilize SR-BI in a manifold manner[63]. First, the initial attachment of HCV particles to SR-BI is independent of E2 but, rather, is mediated by lipoprotein components, such as ApoE[63,64]. Second, the lipid transfer function of SR-BI may facilitate the viral particles access to the next entry step, which would allow exposure of CD81 binding sites on HCV E2 and transfer the virus particle to CD81[63]. Finally, direct interactions between E2 HVR1 and SR-BI enhance infectivity of the particle at post-attachment levels[65].

CD81 is a member of the tetraspanin superfamily defined by four transmembrane domains, a conserved CCG motif, and four cysteine residues that form critical disulfide bonds in the large extracellular loop (LEL). CD81 interacts with HCV E2 glycoprotein *via* a series of discontinuous amino acid residues in the LEL[66]. Several studies suggest that CD81 acts as a post-binding entry molecule. HCV-CD81 interaction induces a conformational rearrangement of the E1 and E2 glycoproteins that facilitates pH-dependent fusion and endocytosis of the virus[67]. Upon CD81 engagement, HCV activates signals allowing the lateral movement of the virus particle/CD81 complex and its delivery to areas of cell-cell contact[68]. Tetraspanins associate with each other to carry out many biological functions, and several CD81 partners and CD81-associated tetraspanins are proposed to play a role in HCV entry[69-71].

CLDN1 is a member of the claudin family of tight junction proteins, consisting of a large and small extracellular loop (EL1 and EL2, respectively), and four transmembrane domains. The highly conserved EL1 is critical for HCV entry[48]. CLDN6 and CLDN9 can replace CLDN1 for HCV entry, but they are expressed only at low levels in the liver[72].

CD81 and CLDN1 act at closely related time-points during HCV entry[73], and direct CD81-CLDN1 interaction is observed[74], strongly suggesting they form a complex to facilitate virus entry. The HCV envelope glycoproteins do not directly interact with CLDN1, but CLDN1 interacts with CD81 and thereby plays an important role during post-binding steps of the HCV entry process[48,73,74]. CLDN1 is expressed both at the basolateral and apical membranes[75]. Interestingly, CLDN1-CD81 complexes are absent from apically located tight junctions, suggesting that virus engagement of basolateral pools of CD81 and CLDN1 may initiate the particle internalization process[75].

EGFR is a receptor tyrosine kinase (RTK) that regulates several key processes, including cell proliferation, survival, and differentiation during development, tissue homeostasis and tumorigenesis[76]. EphA2 mediates cell positioning, cell morphology, polarity and motility[77]. EGFR and EphA2 were recently identified to be required for HCV entry[50] and modulate interactions between CD81 and CLDN1 by EGFR-dependent signaling pathways[78].

OCLN, also belongs to the tight junction protein family, and has been identified as another co-receptor required for a late post-binding event[49]. OCLN is composed of four transmembrane regions, two extracellular loops and N- and C-terminal cytoplasmic regions[79]. The determinants on OCLN of HCV infectivity and species specificity are mapped to the second extracellular loop[80]. Although OCLN has been reported to interact with the HCV E2 glycoproteins[81], whether the interaction is mediated *via* a direct E2-OCLN binding or by indirect interactions with CD81/CLDN1 complexes is unclear.

Internalization of HCV particles is dependent on clathrin-mediated endocytosis[22]. HCV promotes CD81 endocytosis *via* a clathrin- and dynamin-dependent process in association with CLDN1[82].

Recently, TfR1 was identified as a HCV entry factor[51]. TfR1 is ubiquitously expressed in all tissues and is the main receptor for cellular iron uptake into cells *via* clathrin-mediated endocytosis. TfR1 plays a role in HCV infection at the level of glycoprotein-mediated entry, acts after CD81, and possibly is involved in HCV particle internalization[51].

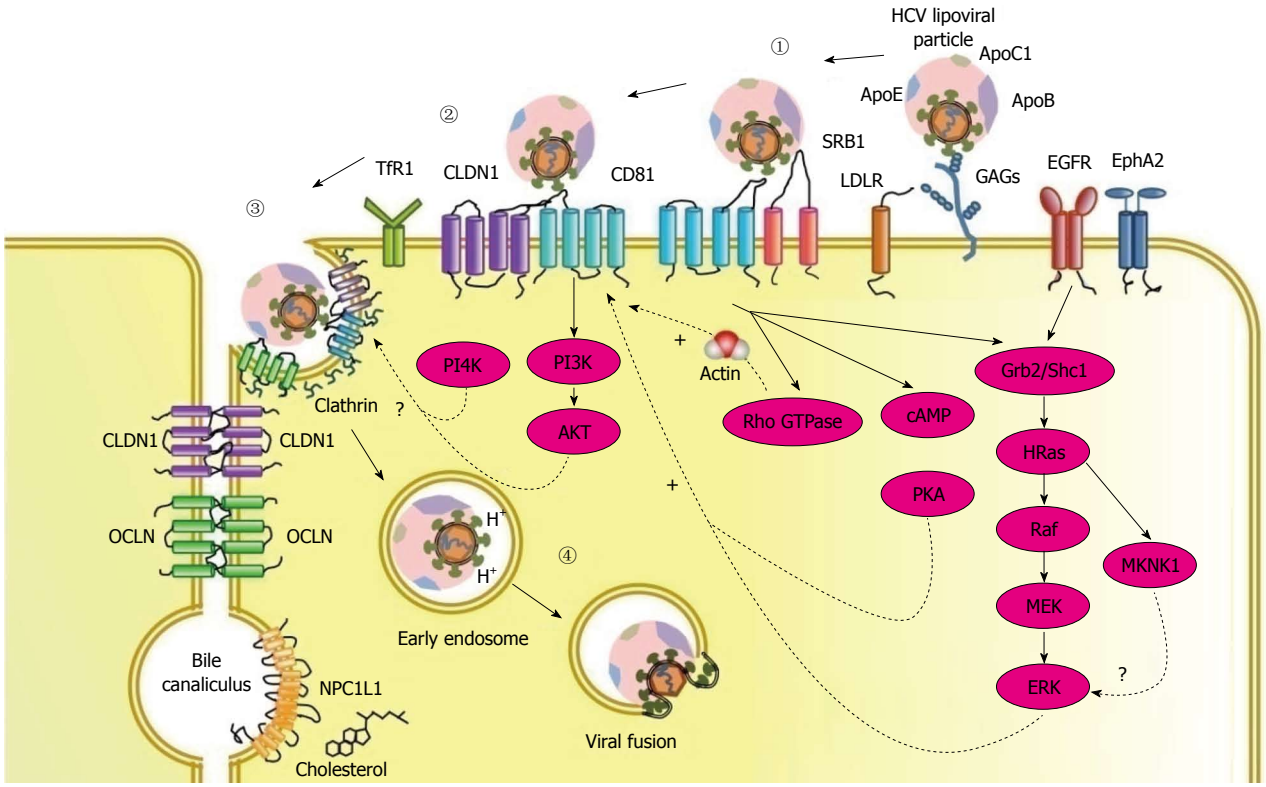

Figure 1 Hepatitis C virus entry into hepatocytes. ① Hepatitis C virus (HCV) LVP binds to glycosaminoglycans (GAGs) and low-density lipoprotein receptor (LDLR), which have high affinity with ApoE. SRBI plays an important role in both binding and post-binding steps through interaction with virion-associated lipoprotein or HCV E2. ② Binding of SRBI to HCV particles allows exposure of CD81 binding sites on HCV E2 and transfer of the virus particles to CD81. Upon interaction with HCV E2, CD81 activates Rho GTPase family members that promote actin remodeling, thus allowing the delivery of the E2-CD81 complex to come into contact with claudin-1 (CLDN1). Two RTKs, epidermal growth factor receptor (EGFR) and EphA2 facilitate the formation of the CD81-CLDN1 complex by the EGFR-activated EGFR/Shc1/Grb2/HRas signaling pathway. The CD81-CLDN1 association is also regulated by PKA in a cAMP-dependent manner to retain CLDN1 on the plasma membrane. MKNK1 was recently identified as a host factor in HCV entry, which possibly acts to facilitate the downstream of EGFR. ③ The virion is primed by the low-pH fusion activity of CD81 and CLDN1 and translocates to the tight junctions in order to be endocytosed. Viral internalization is dependent on clathrin-mediated endocytosis. Junction protein occludin (OCLN) may contribute to this process. TfR1 plays a role in HCV infection at the level of glycoprotein-mediated entry, acts after CD81, and possibly is involved in HCV particle internalization. PI3K-AKT and PI4K pathways are engaged in the late step of HCV entry. However the mechanisms are yet to investigated. ④ Following internalization, HCV fusion occurs in the early endosomes. Low pH environment and virion-associated cholesterol are required for the fusion process. NPC1L1 may play a role in the process by cholesterol transport. After fusion between the viral envelope and an endosomal membrane, the viral genome is released into the cytosol and replication take place.

ENDOSOMAL FUSION

The fusion within the endosomal cell compartment between viral and host membranes is the final step of HCV entry to release the viral RNA for the following HCV life cycle. It is believed that this process is triggered in a receptor-independent but pH-dependent manner, and is closely linked with lipid composition[83]. The virus-receptor interactions and low pH might cause glycoprotein rearrangements to trigger fusion-related protein transition from a pre-fusion state to a post-fusion structure[84].

Of the two envelope protein, E1 acts as a protein chaperone. It has been shown that specific residues of fusion-peptide-like domain on E1 are required for mediating cell fusion and entry of HCV[27,85,86]. Still, the role of this domain in HCV entry needs further investigation. However, the role of E2 in viral fusion is controversial. Previous studies have suggested that a specific region of E2 is a key fusion determinant of HCV[26,27], while Law's team has found no presence of a fusion peptide in HCV E2 by crystal structure analysis[25]. The conformational changes of the envelope proteins finally lead to a fusion pore to release the nucleocapsid into the cytosol[87,88].

Besides the acidic endosomal pH environment, the cholesterol of the target membranes is also found to have a strong promoting effect on the membrane fusion capacity of flaviviruses[89-91]. The NPC1L1 cholesterol uptake receptor has been identified recently to be one of key entry factors for HCV[52]. It has been revealed that it might promote HCV entry by modulating cholesterol homeostasis or influencing cholesterol level of LVPs, thus affecting membrane composition required for membrane fusion[92].

REGULATION OF HCV ENTRY BY HOST KINASES

It has been recently demonstrated that HCV uses host kinases during the entry process to facilitate virus-receptor interaction, and appropriate membrane traffic.

There is strong evidence that CD81 and CLDN-1 work together to facilitate HCV entry. The association of CD81 and CLDN-1 appears to be regulated by mul-

Table 2 Antiviral agents targeting viral entry

Stages of HCV entry process	Target	Antiviral agents	Ref.
HCV particle/ attachment	HCV E1 and E2	Neutralizing antibodies	[102,103]
		Heparin	[41,55]
		Lectins	[106,107]
		EGCG	[109,110]
		Oleanane-type triterpenes	[111]
	Virion-associated lipoprotein	Anti-apoE mAb	[108]
Receptor-mediated endocytosis	SRBI	Anti-SR-BI mAb	[113]
		ITX 5061	[117]
	CD81	Anti-CD81 mAb	[114]
	CLDN1	Anti-CLDN1mAb	[115]
		CLDN1-derived peptide	[116]
	EGFR	Erlotinib	[50]
	NPC1L1	Ezetimibe	[52]
	TfR1	Anti-TfR1 mAb	[51]
	Internalization	Amphipathic DNA polymers	[118]
		Arbidol	[122]
		Tamoxifen	[123]
Endosomal fusion	Fusion	E2-derived peptide	[121]
		CD81-derived peptide	[124]
		Curcumin	[125]
		Phenothiazines	[126]
		Ferroquine	[127]
		aUY11	[128]
		HCV Ⅱ-1/GS-563253	[129]
Regulation	Ras	Tipifarnib	[78]
	PKA	H89	[96]
	MKNK1	RO4475417	[93]
	PI3K	Wortmanin	[99]

Targets within the different stages of Hepatitis C virus (HCV) entry process are depicted, followed by examples of compounds targeting the respective entry step. Stages of development and references are indicated. GAGs: Glycosaminoglycans; CLDN1: Claudin-1; OCLN: Occludin; LDLR: Low-density lipoprotein receptor; EGFR: Epidermal growth factor receptor.

tiple signaling pathways. Using a functional RNAi kinase screen, two RTKs, EGFR and EphA2, are identified as host cofactors for HCV entry by prompting CD81-CLDN1 complex formation[50]. EGFR regulates CD81-CLDN1 co-receptor association by the activation of the EGFR/Shc1/Grb2/HRas signaling pathway[78]. Proteomic analysis has revealed that HRas associates with CD81 and CLDN1, suggesting HRas acts as a molecular switch promoting RTK-mediated HCV entry[78]. Following EGFR stimulation, the Ras/MEK/ERK pathway is activated, which could lead to the activation of MAPK interacting serine/threonine kinase 1 (MKNK1)[93]. MKNK1 was recently identified as a host factor in HCV entry, which possibly acts to facilitate the downstream effect of EGFR[93].

For a multi-receptor virus, successful entry is based on the lateral movement of the virus on the plasma membrane and interaction with sufficient receptors to initiate internalization[94]. In the case of HCV, after the interaction with HCV E2, CD81 activates Rho GTPase family members Rac, Rho and cell division cycle 42 that promote actin remodeling, thus allowing the delivery of the E2-CD81 complex to come into contact with CLDN1[68].

If the localization of either co-receptor is disrupted, the association of CD81 with CLDN1 will be perturbed[95]. By screening a series of kinase inhibitors for their effects on HCV infection, protein kinase A (PKA) was identified as having an important role in HCV entry[96]. Initiation of HCV infection activates PKA in a cAMP-dependent manner to retain CLDN1 on the plasma membrane, which then promotes the entry of HCV[96].

The class I phosphatidylinositol 3-kinase (PI3K) is activated by G-protein-coupled receptors and tyrosine kinase receptors. Upon its activation, it converts phosphatidylinositol 4,5-biphosphate to phosphatidylinositol 3,4,5-triphosphate, which binds to and recruits AKT to the membrane for its phosphorylation. Many flaviviruses regulate the PI3K-AKT pathway for their entry[97,98]. HCV rapidly and transiently activates the PI3K-AKT pathway in the early stage of infection to enhance its entry, and the activation is mediated by the interaction between HCV E2 and its co-receptors, CD81 and CLDN1[99]. However, the exact step of the entry process that the AKT pathway regulates remains elusive.

In addition, phosphatidylinositol 4-kinases type Ⅲ α (PI4KⅢα) and β (PI4KⅢβ) have been suggested to play a role in membrane remodeling and trafficking during HCV entry[100,101]. However, the underlying molecular mechanism is unclear.

HCV ENTRY AS AN ANTIVIRAL TARGET

HCV entry has been revealed as a highly complex process requiring the involvement of viral envelope proteins and multiple host proteins, which offer a large number of promising therapeutic targets (Table 2). Each stage of HCV entry can be designed as an antiviral target, including virus particles, virus attachment, receptor-mediated endocytosis, endosomal fusion, and the regulatory pathways.

HCV entry can be inhibited with monoclonal or polyclonal neutralizing antibodies[102-104]. However, cross-reactive neutralizing antibodies seem to appear later during HCV infection, and several mechanisms contribute to reduce their accessibility to their cognate epitopes. These include the masking of major conserved neutralizing epitopes by HVR1, specific N-linked glycans, and the lipid moiety of the viral particle. Other potential mechanisms of evasion from the neutralizing antibody response include a modulation by HDLs and interfering antibodies, as well as the capacity of the virus to be transferred by cell-to-cell contact[105].

Several non-HCV specific molecules interfering with HCV envelope glycoproteins and abrogating viral attachment have been described. Heparin, a structural analog of GAGs, has been demonstrated to inhibit HCV entry[41,55]. Lectins, such as cyanovirin-N and griffithsin, bind to the high-mannose glycans present on the HCV particles and thereby inhibit HCV entry[106,107]. ApoE antibodies and peptides are able to inhibit HCV entry by

blocking its binding to target cells[108]. Some natural products have been shown to prevent virus binding, such as the green tea polyphenol epigallocatechin-3-gallate[109,110] and oleanane-type trierpene[111].

To overcome the high variability of the viral envelope proteins, small-molecule host-targeting agents (HTAs) are also being investigated[112]. Antibodies or peptides targeting CD81, SR-B1 and CLDN1 have been shown to be an efficacious way to prevent HCV infection both *in vitro* and *in vivo* in a genotype-independent manner[113-116]. ITX 5061, one of the most promising HTAs, which promotes HDL levels in animals and patients by targeting SRB1[117], is currently in phase 2 clinical trials. Clathrin-dependent endocytosis[118-120] and endosomal fusion[121] are also potential targets for the development of anti-HCV compounds. Arbidol suppresses clathrin-mediated endocytosis by hindering HCV endosomal trafficking and impairing dynamin-2-induced membrane scission[122]. Tamoxifen is a selective estrogen receptor modulator, and affects both viral binding and post-binding events including endocytosis[123]. The stapled peptides are designed according to the linear peptide sequence of the large extracellular loop of CD81, which has been reported to play an important role in HCV E2 binding interaction, and has an inhibitory effect on HCV membrane fusion[124]. Curcumin affects membrane fluidity, therefore impeding viral binding and fusion[125]. Phenothiazines inhibit HCV infection at an early stage of the viral cycle by interfering with virion-cell fusion[126]. They insert into cholesterol-rich domains of target membranes and perturb cholesterol distribution in lipid membranes, creating a barrier to virion-cell fusion. Ferroquine, an anolog of chloroquine, suppresses HCV infection at a late post-binding step by impairing the fusion process[127]. An arabino-based rigid amphipathic fusion inhibitor, aUY11, inhibits HCV infection at the early fusion process by interacting with envelope lipids[128]. HCV-II/GS-563253 suppresses HCV endosomal fusion by locking the viral envelope pre-fusion state or formation of an incapable envelope conformation for viral fusion[129].

Given the relevance of host cell kinases for HCV entry and the number of kinase inhibitors being developed to treat a wide variety of human diseases, kinase inhibitors have been suggested as a novel class of antivirals for the prevention and treatment of HCV infection[78,93,96,99]. Erlotinib, a clinically approved inhibitor of EGFR, and the NPC1L1 inhibitor ezetimibe have been shown to impair HCV infection *in vivo*[50,52].

CONCLUSION

The importance of HCV entry owes to its critical role in the initiation of infection, the major target of immune responses and the determination of tissue and species tropism[24]. In recent years, substantial progress has been made to decipher the mystery of HCV entry. Current data support a complex interplay between the HCV-encoded glycoproteins E1 and E2 and four receptors - SR-BI, CD81, and tight junction proteins CLDN1 and OCLN in defining HCV entry. EGFR, NPC1L1 and TfR1 are important cofactors for HCV entry. HCV entry has been revealed as a highly complex process requiring orchestration of several cell surface molecules and regulation of multiple host kinases, which offer a multitude of promising targets for antivirals.

The ideal therapeutic regimen is thought to be an all-oral, IFN-free combination cocktail with pan-genotype coverage, minimal side effects and high virological cure rates in all patient groups[1]. Given the chronic nature and genetic diversity of HCV, targeting host entry factors with antibodies or small-molecule inhibitors could block the spread of DAA-resistant variants and disturb infection dynamics necessary to maintain chronic liver infection. The most advanced inhibitor of HCV entry is ITX 5061, a small molecule compound that was initially identified to promote HDL levels in animals and patients by targeting SRBI.

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