Syndecan-1 Serves as the Major Receptor for Attachment of Hepatitis C Virus to the Surfaces of Hepatocytes

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Our recent studies demonstrated that apolipoprotein E mediates cell attachment of hepatitis C virus (HCV) through interactions with the cell surface heparan sulfate (HS). HS is known to covalently attach to core proteins to form heparan sulfate proteoglycans (HSPGs) on the cell surface. The HSPG core proteins include the membrane-spanning syndecans (SDCs), the lycosylphatidylinositol-linked glypicans (GPCs), the basement membrane proteoglycan perlecain (HSPG2), and agrin. By the present study, we have profiled each of the HSPG core proteins in HCV attachment. Substantial evidence derived from our studies demonstrates that SDC1 is the major receptor protein for HCV attachment. The knockdown of SDC1 expression by small interfering RNA (siRNA)-induced gene silence resulted in a significant reduction of HCV attachment to Huh-7.5 cells and stem cell-differentiated human hepatocytes. The absence of SDC2 expression also caused a modest decrease of HCV attachment. In contrast, the siRNA-mediated knockdown of other SDCs, GPCs, HSPG2, and agrin had no effect on HCV attachment. More importantly, ectopic expression of SDC1 was able to completely restore HCV attachment to Huh-7.5 cells in which the endogenous SDC1 expression was silenced by specific siRNAs. Interestingly, mouse SDC1 is also fully functional in mediating HCV attachment when expressed in the SDC1-deficient cells, consistent with recent reports that mouse hepatocytes are also susceptible to HCV infection when expressing other key HCV receptors. Collectively, our findings demonstrate that SDC1 serves as the major receptor protein for HCV attachment to cells, providing another potential target for discovery and development of antiviral drugs against HCV.
only apolipoprotein required for HCV production in nonhepatic 293T cells (41). Apart from its role in HCV assembly, apoE also mediates HCV attachment through its N-terminal receptor-binding domain, which binds the cell surface receptors HSPGs (32) (J. Jiang and G. Luo, unpublished results). Removal of HS from cell surface HSPGs by pretreatment of cells with heparinases could efficiently prevent HCV binding and infection (24–26, 32). HS is covalently attached to core proteins to form HSPGs. The HSPG core proteins include the membrane-spanning syndecans (SDCs), the lycosphosphatidylinositol-linked glypicans (GPCs), the basement membrane proteoglycan perlecan (HSPG2), and agrin (AGRN) (42, 43). However, the core proteins of HSPGs required for HCV attachment in human hepatocytes have not been defined. In this study, we have profiled the importance of each of the HSPG core proteins in HCV attachment. Our findings demonstrate that syndecan-1 (SDC1) is the major receptor protein for HCV attachment to cell surface. Both human and mouse SDC1 are fully functional to mediate the attachment of HCV to target cells, providing another potential molecular target for discovery and development of anti-HCV drugs.

MATERIALS AND METHODS

Viruses and cell culture. The cell culture-adapted HCV of genotype 2a (JFH1) was grown in Huh-7.5 cells, as used in our earlier work (43, 78). This cell culture-grown HCV is designated HCVcc. The genotype 1b HCV was obtained from hepatitis C patients (TeraGenix, Fort Lauderdale, FL) and was generously provided by Hengli Tang (44). The clinical 1b isolate is designated HCV1b. Huh-7.5 and adenovirus packaging cell line AD293 cells (Agilent Technologies) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 0.1 mM nonessential amino acids (Sigma), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a 5% CO2 incubator. Human embryonic stem cell (hESC) line WA09 (H9) was obtained from the WiCell Research Institute and maintained on Gelrex-coated culture plates in Stem Pro medium (Invitrogen, Carlsbad, CA). hESC-differentiated human hepatocytes (DHHs) were prepared and maintained as described previously (44).

Antibodies and synthetic siRNAs. HCV NSSA monoclonal antibody (Mab) (9E10) was provided by Charlie Rice. Rabbit anti-NSSA monoclonal antibody and goat anti-rabbit secondary antibody conjugated with Alexa 488 were from Virogen and Invitrogen, respectively. β-Actin monoclonal antibody (AC15) was from Sigma-Aldrich. Human SDC1 monoclonal and polyclonal antibodies (sc-12765, 553712, and 550804) were purchased from Santa Cruz and BD Biosciences, respectively. SDC2-specific monoclonal antibody (2965) was from R&D Systems. NS3-specific monoclonal antibody was produced in the lab as previously described (45). CD81 Mab (clone 5A6) was from Santa Cruz. Claudin-1 monoclonal antibody and occludin-specific rabbit polyclonal antibody were from Invitrogen. SR-B1 rabbit polyclonal antibody (EP15565) was from Abcam. Horseradish peroxidase-conjugated goat anti-mouse IgG was from Pierce. Cellular gene-specific small interfering RNAs (siRNAs) (SmartPool and individual siRNAs) and nonspecific control (NCS) siRNA were synthesized by Dharmacon.

Silencing of cellular gene expression by RNA interference (RNAi). Cell culture plates (12- and 24-well plates) were coated with 50 μg/ml of collagen (Huh-7.5) or Gelretx (DHHs). Huh-7.5 cells and DHHs were seeded overnight at 37°C in an incubator and then transfected with various concentrations (0, 2, 10, and 50 nM) of siRNAs using RNAiMax transfection reagent (Invitrogen), as previously described (32). An NSC siRNA was used as a negative control. At 48 h posttransfection (p.t.), the mRNA and protein levels of targeted cellular genes were determined by a quantitative reverse transcription-PCR (qRT-PCR) method and Western blotting using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin as internal controls, respectively.

HCV attachment and infection assays. At 48 h after siRNA transfection, Huh-7.5 cells and DHHs in 12-well cell culture plates were incubated with HCVcc at a multiplicity of infection (MOI) of 10 on ice for 2 h or at 37°C for 1 h or with HCV1b at 37°C for 2 h (attachment assay). The unbound HCV was removed by washing with 1× phosphate-buffered saline (PBS) three times. Total RNA was extracted with RNAzol reagent (Molecular Research Center) and was used for quantification of HCV virion RNA (vRNA) by a real-time qRT-PCR method. HCV infection was carried out by incubation of HCVcc with Huh-7.5 cells at 37°C for 1.5 h, and the HCV-infected cells were washed with PBS and incubated with fresh DMEM at 37°C for 24 h (single-cycle growth). The cell culture supernatants were collected for determination of infectious HCV titers by limiting dilution, whereas the HCV-infected Huh-7.5 cells were lysed in radioimmunoprecipitation assay (RIPA) buffer for detection of HCV NSSA and cellular proteins by Western blotting using specific monoclonal antibodies.

Quantification of HCV vRNA and cellular gene mRNAs by one-step or two-step qRT-PCR method. The levels of HCV vRNA and cellular gene mRNAs were determined by either one-step qRT-PCR or the two-step RT and qPCR method using specific primers and probes (available on request) and the StepOnePlus real-time PCR system (Applied Biosystems). The one-step real-time qRT-PCR was carried out using the following program: 50°C for 10 min and 95°C for 5 min followed by 40 cycles at 95°C for 10 s and 60°C for 30 s. For two-step qRT-PCR, cDNAs were first synthesized from total RNA using Moloney murine leukemia virus (M-MuLV) reverse transcriptase (New England Biolabs) for GPCs, HSPG2, and AGRN, and then qPCR was carried out as follows: 10 min at 95°C and 40 cycles at 95°C for 15 s and 60°C for 1 min. GAPDH was used as a control to normalize total amounts of RNAs used in qRT-PCR.

Western blotting. The protein concentration of cell lysates was determined using a protein assay reagent (Bio-Rad). Twenty micrograms of total protein for each sample was resolved by 4% paraformaldehyde solution and permeabilized with 0.1% Triton X-100, as previously described (46). The NS3 protein in the HCV-infected Huh-7.5 cells was reacted with an NS3-specific monoclonal antibody and visualized with the secondary donkey anti-mouse antibody conjugated with Alexa Fluor 594 fluorescein (Molecular Probes). The cell culture supernatants was titrated by 10-fold serial dilution. The serially diluted HCV was used to infect Huh-7.5 cells in 96-well plates. After 2 h of incubation at 37°C, 100 μl of fresh DMEM containing 1% methylcellulose was added into each well. At 48 h postinfection (p.i.), focus-forming units (FFU) were determined by IFA staining using an NS3-specific monoclonal antibody as described previously (36). Infectious HCV titer was calculated based on the average number of NS3-positive FFU/ml in triplicate assays.

Construction of recombinant adenoviruses expressing syndecans. To ectopically express human and mouse SDC1 that is resistant to RNA interference (RNAi), three silent nucleotide mutations were introduced into the siRNA-targeting region. Thus, the SDC1-specific siRNA used in this study would silence only endogenous but not ectopic SDC1 expression. Silent mutations were introduced into human and mouse SDC1 genes by two overlapping PCR methods using synthetic oligonucleotide primers. PCR DNA fragments were cloned into pShuttle-CMV/hSDC1.
Ectopic expression of SDC1 in Huh-7.5 cells with silencing of endogenous SDC1. Huh-7.5 cells in 12-well plates were transfected with 0.2 nmol of SDC1-specific siRNA, whose target sequence is CCAACACGGA GGAUUUCUA, and NSC siRNA at 37°C for 24 h. The siRNA-transfected cells were infected with recombinant adenoviruses expressing hSDC1m or mSDC1m at an MOI of 10 at 37°C for 6 h. The unbound adenoviruses were removed, and fresh DMEM was added to each well. At 24 h p.i., Huh-7.5 cells were infected with HCVcc at an MOI of 10 at 37°C for 6 h. The unbound adenoviruses were removed and fresh DMEM was added to each well. At 24 h p.i., Huh-7.5 cells in 12-well plates were transfected with 0.2 nmol of SDC1-specific siRNA, whose target sequence is CCAACACGGA GGAUUUCUA, and NSC siRNA at 37°C for 24 h. The siRNA-transfected cells were infected with recombinant adenoviruses expressing hSDC1m or mSDC1m at an MOI of 10 at 37°C for 6 h. The unbound adenoviruses were removed, and fresh DMEM was added to each well. At 24 h p.i., Huh-7.5 cells were infected with HCVcc at an MOI of 10 at 37°C for 1 h. The unbound HCVcc was removed by washing cells with 1× PBS three times. Total RNA was extracted with RNAzol reagent and was used for determination of HCV vRNA and SDC1 mRNA levels by qRT-PCR methods.

Statistical analysis. All data were analyzed by one-way analysis of variance (ANOVA) using SPSS 20.0 software. A P value of <0.05 was considered a statistically significant difference.

RESULTS
Profiling of the HSPG core proteins in HCV attachment. Previous studies by others suggested that HSPGs are important for HCV infection in cell culture (24–26). Our recent studies demonstrated that apoE mediates HCV attachment through interaction with the cell surface heparan sulfate (HS) (32). HS is covalently attached to core proteins of HSPGs, which include SDCs, GPCs, HSPG2, and AGRN, serving as core proteins for HSPG synthesis and modification. To determine the importance of each of these HSPG core proteins in HCV attachment, we carried out an initial profiling of HSPG core protein genes using synthetic siRNAs specific to each of the SDC, GPC, HSPG2, and AGRN mRNAs. A mix of 4 individual (SmartPool) siRNAs targeting different regions of each mRNA was used to specifically silence the expression of SDC1, SDC2, SDC3, SDC4, GPC1, GPC2, GPC3, GPC4, GPC5, GPC6, HSPG2, and AGRN individually in Huh-7.5 cells. The knockdown of gene expression was determined by quantifying the levels of mRNAs using a real-time qRT-PCR method and specific primers. The mRNAs encoding 12 HSPG core proteins were efficiently reduced in a dose-dependent manner compared to that of nonspecific control siRNA (Fig. 1). Specific siRNAs at a 50 nM concentration resulted in more than 90% reduction of SDC1, SDC2, SDC3, and SDC4 mRNAs, respectively (Fig. 1A). Likewise,
the levels of SDC1 and SDC2 proteins were proportionally decreased by increasing concentrations of siRNAs, resulting in >80% reduction of SDC1 and undetectable SDC2 expression at 50 nM specific siRNAs (see Fig. 3A). Similarly, the levels of GPC1, GPC2, GPC3, GPC4, GPC5, GPC6, and HSPG2 mRNAs were decreased by >80% at a 50 nM siRNA concentration. To a lesser extent, AGRN mRNA was lowered by 60% at 50 nM siRNAs (Fig. 1B).

The effects of siRNA-mediated knockdown of HSPG core protein expression on HCVcc attachment were subsequently determined. At 48 h after siRNA transfection, Huh-7.5 cells were incubated with HCVcc on ice for 2 h, under which conditions HCV can bind but is unable to enter cells. Unbound virus was completely removed by extensive washing. The RNA of cell-attached HCVcc was then extracted and quantified by a real-time qRT-PCR method using specific primers and probes. Interestingly, knockdown of SDC1 expression remarkably decreased the levels of HCV vRNA in a dose-dependent manner. The SDC1-specific siRNAs at 2, 10, and 50 nM concentrations resulted in about 50%, 75%, and 85% reductions of HCV vRNA, respectively (Fig. 1C). The degree of reduction of HCV attachment is consistent with the knockdown of SDC1 expression at corresponding siRNA concentrations (Fig. 1A). SDC2 siRNAs also caused a modest but significant decrease of HCV vRNA with 50% reduction of HCVcc attachment at 50 nM siRNAs. However, SDC3 and SDC4 siRNAs did not significantly affect the levels of HCV vRNA (Fig. 1C). None of the GPC, HSPG2, and AGRN siRNAs had significant inhibition of HCVcc attachment, although they efficiently silenced the expression of their target genes, similarly to SDCs (Fig. 1B and D).

**Effects of the siRNA-mediated silencing of SDC1 and SDC2 expression on HCVcc attachment and infection.** To confirm the importance of SDC1 and SDC2 in HCV attachment, we carried out HCVcc attachment and infection experiments at 37°C. Similarly to that observed at 4°C, knockdown of SDC1 expression efficiently prevented HCVcc from attaching to Huh-7.5 cells when tested at 37°C, decreasing HCVcc attachment by more than 70% at 50 nM siRNAs (Fig. 2). However, knockdown of SDC2 had only a modest (<30%) effect on HCV attachment. The silencing of SDC3 and SDC4 expression did not affect HCV attachment. These results are in line with the data obtained from an HCVcc attachment assay at 4°C (Fig. 1C). Next, we determined the effects of the siRNA-induced SDC1 and SDC2 knockdown on HCV infection. Huh-7.5 cells were transfected with various amounts of SDC1 siRNAs, SDC2 siRNAs, or NSC siRNA and then infected with HCVcc at 37°C for 1.5 h. At 24 h p.i., the levels of SDC1, SDC2, and HCV NS5A proteins were determined by Western blotting. Also, the infectious HCV titers in the cell culture supernatants were quantified by limiting dilution. The levels of SDC1 and SDC2 expression were proportionally decreased with increasing amounts of specific siRNAs, which caused a >80% reduction of SDC1 expression and an undetectable level of SDC2 at 50 nM concentrations of corresponding siRNAs. More importantly, the silencing of SDC1 expression resulted in a dose-dependent reduction of NS5A, which is in parallel with the decrease of SDC1 expression (Fig. 3A). When Huh-7.5 cells were infected with HCVcc and then cotransfected with SDC1 siRNAs, the levels of NS5A remained unchanged, although SDC1 was efficiently silenced, suggesting that SDC1 siRNA did not affect viral RNA replication (data not shown). However, the levels of NS5A were not significantly affected by SDC2 siRNAs at 2 and 10 nM concentrations, even though SDC2 expression was lowered by 60% and 74%, respectively. SDC2 siRNA at a 50 nM concentration modestly reduced the level of NS5A (Fig. 3A), which is consistent with its effect on HCV vRNA (Fig. 2). Similarly, infectious HCV titers were reduced by SDC1-specific siRNA in a dose-dependent manner, resulting in a nearly 100-fold reduction at a 50 nM concentration of siRNAs (Fig. 3B). Again, the silence of SDC2 expression caused only a modest reduction at 50 nM but not at 2 nM and 10 nM concentrations (Fig. 3B), similarly to NS5A reduction (Fig. 3A). As expected, neither SDC1 and SDC2 expression nor HCV infection was affected by NSC siRNA (Fig. 3). Taken together, these data suggest that SDC1 likely serves as the major receptor protein for HCV attachment during virus infection.

To further validate the physiological relevance of SDC1 in HCV attachment, we used a clinical isolate of genotype 1b HCV (HCV1b) obtained from hepatitis C patients together with DDHs in an HCV attachment assay. Unlike Huh-7.5 cells, DDHs resemble primary human hepatocytes (PHHs) and are susceptible to infection by clinical HCV isolates, as demonstrated previously by others (44, 47). To confirm that DDHs prepared in our own lab were susceptible to HCV infection, DDHs at day 10 were infected with HCVcc. NS5A in the HCVcc-infected DDHs was stained using an NS5A-specific polyclonal antibody. Similarly to previous findings (44), most DDHs were permissive to HCV infection (Fig. 4A). However, a minor fraction of cells did not stain positively for NS5A, suggesting that these cells were not fully differentiated to render them permissive to HCV infection (Fig. 4A), consistent with previous observations (44). Subsequently, the effects of siRNA-mediated SDC1 silencing on HCV1b attachment were determined. Like Huh-7.5 cells (Fig. 1C and Fig. 2), SDC1 mRNA in DDHs was proportionally silenced by increasing concentrations of specific siRNAs with an about 80% reduction of SDC1 mRNA at 50 nM SDC1-specific siRNA (Fig. 4B). Consequently, knockdown of SDC1 expression potently suppressed the attachment of HCV1b to DDHs, resulting in more than 70% reduction of HCV1b attachment to DDHs at a 50 nM concentration.
(Fig. 4C). The inhibition of HCV1b attachment by SDC1 siRNA correlated with the decrease of SDC1 mRNA in DHHs (Fig. 4B). As a control, NSC siRNA did not affect the levels of SDC1 mRNA and HCV1b attachment in DHHs (Fig. 4). Collectively, these findings demonstrate that SDC1 is important for HCV attachment during virus infection.

Restoration of HCV attachment by ectopic expression of SDC1. To exclude possible off-target effects associated with RNA interference (RNAi), we sought to determine whether ectopic expression of SDC1 would restore HCV attachment to Huh-7.5 cells defective in endogenous SDC1 expression. The endogenous SDC1 expression was silenced by transfection with a synthetic siRNA, which was found to most potently silence SDC1 expression among 4 individual SmartPool siRNAs profiled (Fig. 5 and data not shown). Unlike SmartPool siRNAs, the individual siRNA was less able to induce degradation of endogenous SDC1 mRNA. Therefore, higher concentrations of the SDC1 siRNA were required to efficiently silence endogenous SDC1 expression, with an about 80% reduction of SDC1 mRNA and protein at a 200 nM concentration (Fig. 5). To ectopically express the siRNA-resistant SDC1, three synonymous nucleotide mutations were introduced into the siRNA-targeting region of either the human or the mouse SDC1 gene. These mutations do not cause any change of amino acids but instead create nucleotide mismatches between SDC1 mRNA target and siRNA sequences. Therefore, the SDC1-specific siRNA could silence endogenous but not ectopic SDC1 expression. Recombinant adenoviruses were constructed to ectopically express human and mouse SDC1s. A nonspecific control siRNA (siNSC) and a recombinant adenovirus expressing a lacZ gene (LacZ) were used as negative controls in these experiments (Fig. 5). Huh-7.5 cells were transfected with SDC1-specific siRNA and then infected with recombinant adenoviruses. After 24 h, HCV attachment experiments were carried out at 37°C for 1 h. Upon extensive washing to remove unbound HCV, total RNAs were extracted from the HCVcc-attached cells. The levels of both SDC1 mRNA and HCV vRNA were determined by real-time qRT-PCR methods. Results show that SDC1 was ectopically expressed in the siRNA-transfected Huh-7.5 cells as shown by mRNA quantification (Fig. 5C) as well as SDC1 protein expression detected by Western blotting (Fig. 5D). More significantly, ectopic expression of both human and mouse SDC1s in Huh-7.5 cells fully restored HCV attachment, which otherwise was inhibited by knockdown of endogenous SDC1 expression (Fig. 5E). In fact, overexpression of human SDC1 significantly increased the susceptibility of Huh-7.5 cells to HCVcc attachment (Fig. 5E). Additionally, infectious HCV titers in the culture supernatants were also completely restored (data not shown). Altogether, these data demonstrate that SDC1 functions as the major receptor for HCV attachment.

Effect of SDC1 silencing on the expression of CD81, CLDN1, OCLN, and SR-B1. To exclude a possible effect of SDC1 siRNAs on the expression of key HCV receptors such as CD81, CLDN1, OCLN, and SR-B1, we determined their respective expression levels by Western blotting in the SDC1 siRNA-transfected Huh-7.5 cells in the same way as that shown in Fig. 3A. None of these key HCV receptors was significantly affected by the siRNA-mediated knockdown of SDC1 expression (Fig. 6), suggesting that reduction of HCV attachment by silencing SDC1 expression was not due to its effect on CD81, CLDN1, OCLN, and SR-B1 expression. In fact, our previous studies demonstrated that the aforementioned HCV receptors do not play a significant role in HCV attachment, although they are critically important for HCV infection at postattachment steps (32).

DISCUSSION

A number of previous studies suggested that HSPGs serve as receptors to mediate initial binding of many different viruses to the cell surface, including but not limited to herpes simplex virus 1 (HSV-1); human papillomavirus (HPV); hepatitis B, C, and E viruses; rotavirus; and respiratory syncytial virus (48–64). Compelling evidence accumulated over the years suggests that the heparan sulfate covalently attached to core proteins of HSPGs is required for mediating virus attachment to the cell surface. In the case of HCV, either removal of HS from the cell surface by heparinas or addition of heparin and its derivatives could efficiently...
block HCV infection (24–26, 32). However, the importance and underlying mechanism of the HSPG core proteins in HCV attachment have not been experimentally examined. In the present study, we have profiled each of the 12 HSPG core proteins using gene-specific siRNAs and an in vitro HCV attachment assay. Findings derived from our experiments suggest that SDC1 acts as the major receptor protein for binding of HCV to hepatocytes. Knockdown of SDC1 expression by specific siRNAs in Huh-7.5 and DHH cells blocked HCV attachment by over 80% (Fig. 1C, 2, and 4). Consistently, the siRNA-induced silence of SDC1 expression also resulted in reduction of HCV infection and production (Fig. 3). More significantly, the siRNA-induced downregulation of SDC1 expression also blocked the binding of a clinical HCV of genotype 1b to DHH cells (Fig. 4), suggesting that SDC1 likely functions as the receptor for HCV attachment in vivo. DHHs resemble primary human hepatocytes in many aspects as revealed by recent studies (44, 47). More importantly, ectopic expression of SDC1 not only fully restored but actually enhanced the susceptibility of Huh-7.5 cells to HCV attachment (Fig. 5D). It should be noted that knockdown of SDC2 expression also resulted in a modest but not dose-dependent inhibition of HCV attachment (Fig. 1C and 2). This may explain why the binding of HCV to the cell surface was not completely blocked by knockdown of SDC1 expression per se. However, other HSPG core proteins such as SDC3, SDC4, GPCs, HSPG2, and ARGN do not appear to play a significant role in HCV attachment, since knockdown of their expression had little effect on HCV binding (Fig. 1 and 2). Therefore, it is most likely that SDC1 proteoglycan serves as the major receptor for mediating initial binding of HCV to hepatocytes before sequential interactions with other key receptors and/or coreceptors. Future studies are warranted to corroborate the physiological significance of SDC1 in HCV infection in vivo. A fully humanized mouse model of HCV infection and replication made it possible to determine the biological relevance of SDC1 in HCV infection using wild-type and SDC1-knockout mice (65). Our results clearly show that ectopic expression of a mouse SDC1 was able to fully restore the susceptibility of Huh-7.5 cells to HCV binding, consistent with the susceptibility of mouse hepatocytes to HCV infection in vivo (65). It is noteworthy that SDC1 expression was detected in both mouse hepatoma cell line Hepa 1-6 and DHHs at about 40% of that in Huh-7.5 cells, consistent with the important role of SDC1 in HCV attachment in the cell types tested in this study (data not shown).

Why SDC1 but no other HSPG core proteins are preferentially
utilized by HCV for initial attachment to hepatocytes remains unknown. Previous studies found that different viruses favor distinct syndecans as attachment receptors. For instance, dengue virus, another member of the Flaviviridae family, prefers SDC2 proteoglycan as its binding receptor (66). For human immunodeficiency virus type 1 (HIV-1), SDC3 was found to be a major attachment receptor on dendritic cells (DCs) (67). However, SDC1, SDC2, SDC3, and SDC4 could all serve as HIV-1 attachment receptors when ectopically expressed in nonpermissive cells (68). In the case of HPV, SDC1 appeared to favorably increase cell permissiveness to the binding of virus-like particles and authentic virions compared with SDC4 and GPC1 expressed ectopically (69). Thus, the choice of different HSPG core proteins as attachment receptors for different viruses is complex and is
likely influenced by multiple factors. The levels of HSPG core protein expression among different cell types may contribute to their usage as attachment receptors by different viruses. More importantly, the HS attachment was found to be critically important for the function of SDCs. There are three highly conserved sites available for HS attachment at the N-terminal ectodomain of SDCs. HS attachment to each of these serine residues individually or in different combinations at different cell types may determine the tropism of SDCs as attachment receptors for different viruses. The SDC1 detected in mouse and human hepatocytes appears as a homogenous proteoglycan with a mass of >80 kDa, suggesting that it is highly sulfated in hepatocytes (Fig. 5B and D). We have tested a couple of commercially available SDC1-specific monoclonal and polyclonal antibodies in the HCV attachment assay. However, none of the antibodies examined had any effect on HCV attachment (data not shown). This could be due to their lack of blocking activity. Alternatively, HS moieties attached to SDC1 are the determinants for binding of protein ligands. It is known that specific interaction between HS and protein ligands depends on unique oligosaccharide sequences containing iduronic acid, N-sulfated glucosamine residues, and O-sulfated sugars attached to the core proteins (71). The saccharides can be modified by N and O sulfation at different positions in the cell (72). It was found that 3-O-sulfation of specific glucosamine residues in HS is important for HCV-1 binding (73). Therefore, various derivatives of HS add another complex to the selection of SDCs as attachment receptors for different viruses. Whether the ectodomain amino acid residues of SDCs play any role in virus attachment has not been determined. The amino acid residues of ectodomains are divergent between SDC1, SDC2, SDC3, and SDC4, although their C-terminal membrane-anchorage domains are highly conserved. However, human and mouse SDC1 proteins are nearly identical, differing in only three residues. This may explain why both human and mouse SDC1 have the same capacity of mediating HCV attachment to hepatocytes (Fig. 5).

It was suggested that HCV envelope protein E2 mediates the initial binding of HCV to the cell surface through interactions with HSPGs (24). However, substantial evidence derived from our studies and studies by others demonstrates that apoE anchored on the envelope of HCV mediates HCV attachment by binding to the cell surface HSPGs (32–36, 40, 74). apoE- but not HCV E2-specific monoclonal antibody was found to efficiently block HCV attachment (32). Additionally, short peptides derived from the apoE receptor-binding region could potently block HCV attachment to cells (32, 74). Similarly, mutations at the apoE receptor-binding domain resulting in defects in HCV infectivity were found to inactivate the ability of apoE to bind to heparin as shown by an in vitro heparin pulldown assay (32). It is believed that the 2-O-sulfate groups of the iduronic acid monosaccharides or the N- and 6-O-sulfate groups of the glucosamine sulfate monosaccharides are important for apoE binding (75). However, which GAG and GAG modification are required for apoE binding remains unknown. Future studies are warranted to determine the importance of HS side chains in HCV attachment.

Identification of SDC1 as the major receptor for HCV attachment provides a potential molecular target for antiviral drug discovery and development against HCV. The proof of principle for SDC1 as a viable target was first demonstrated by the findings that a recombinant SDC1–Fc fusion protein expressed in CHO cells could potently block HIV infection of T cells, macrophages, and dendritic cells as well as HSV-1 infection (76). Recently, 3-O-sulfonated HS octasaccharides were synthesized and were shown to have antiviral activity inhibiting HSV-1 infection (77). It is anticipated that recombinant SDCs, HS mimetics, and/or small-molecule inhibitors can be developed as prophylactic and/or therapeutic agents for different viruses.

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