

Another Putative Receptor for Hepatitis C Virus

Scarselli E, Ansuini H, Cerino R, Roccasecca RM, Acali S, Filocamo G, Traboni C, Nicosia A, Cortese R, Vitelli A. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002;21:5017-5025. Reprinted by permission of Oxford University Press.

Abstract

We discovered that the hepatitis C virus (HCV) envelope glycoprotein E2 binds to human hepatoma cell lines independently of the previously proposed HCV receptor CD81. Comparative binding studies using recombinant E2 from the most prevalent 1a and 1b genotypes revealed that E2 recognition by hepatoma cells is independent from the viral isolate, while E2-CD81 interaction is isolate specific. Binding of soluble E2 to human hepatoma cells was impaired by deletion of the hypervariable region 1 (HVR1), but the wild-type phenotype was recovered by introducing a compensatory mutation reported previously to rescue infectivity of an HVR1-deleted HCV infectious clone. We have identified the receptor responsible for E2 binding to human hepatic cells as the human scavenger receptor class B type I (SR-BI). E2-SR-BI interaction is very selective since neither mouse SR-BI nor the closely related human scavenger receptor CD36, were able to bind E2. Finally, E2 recognition by SR-BI was competed out in an isolate-specific manner both on the hepatoma cell line and on the human SR-BI-transfected cell line by an anti-HVR1 monoclonal antibody.

Comments

Infection of cells by viruses begins with the attachment of the virion to the surface of the host cell. Attachment is mediated by the binding of a protein present at the surface of the native virion to a molecule on the cell surface acting as a virus receptor. In addition to primary binding receptors, some viruses also interact with secondary receptors (coreceptors) that bind either to native virions or instead to altered forms of virions that are produced as a result of the primary binding step. Virus receptors and coreceptors are potentially any normal component associated with the host cell membrane. Because of their critical involvement in the early steps of infection, viral receptors and coreceptors are major determinants of viral tropism. However, their expression may not necessarily be the sole determinant for viral infection.

The envelope glycoprotein complex E1-E2 is the viral component that is supposed to be present at the surface of hepatitis C virus (HCV) particles,¹ and it is therefore the obvious ligand candidate for cellular receptors. Alternatively, because of the physical association of HCV with low- or very low-density lipoproteins (LDL or VLDL) in serum, the LDL receptor has been proposed as a candidate receptor for HCV.^{2,3} The LDL receptor has been reported to mediate HCV internalization via binding to virion-associated LDL particles,² but there is no available experimental data indicating that such interactions lead to a productive infection.

In the absence of a suitable cell culture system for HCV, an alternative approach to search for candidate receptors has been to use truncated soluble forms of HCV glycoproteins for binding studies to human cells.⁴ By using a soluble form of E2 as a probe, CD81 has been identified as another putative receptor for HCV,⁵ and, by a very similar approach, Scarselli et al. have identified a third candidate receptor for HCV. The identification of the latter followed the observation that human hepatocarcinoma cells HepG2 do not express CD81 on their surface but they are able to efficiently bind E2, suggesting that a cell surface molecule other than CD81 is responsible for E2 binding.

A purification approach by cross-linking E2 to this new putative receptor has identified an 82 kd glycoprotein interacting with soluble E2. To identify the HepG2 receptor responsible for E2 binding, the investigators have looked for the compartmentalization of this protein in the plasma membrane. They have observed a reduction of E2 binding after incubating HepG2 cells with β -methyl cyclodextrin, a compound that selectively removes cholesterol from membranes and affects the function of membrane proteins located in the so-called "lipid rafts." Lipid rafts are membrane microdomains enriched in cholesterol and sphingolipids in the exoplasmic leaflet of the membrane bilayer.⁶ Due to their specific composition, lipid rafts segregate and concentrate distinct classes of proteins. Since the number of known proteins located in lipid rafts is quite limited, the scavenger receptor class B type I (SR-BI) was easily identified as the candidate molecule by the use of specific antibodies. This was confirmed by the observation that human SR-BI expression in CHO cells confers the ability to bind soluble E2. E2-SR-BI interaction is very selective because no binding of E2 was

observed on CHO cells expressing CD36, another member of the scavenger receptor family with whom SR-BI shares many ligands.⁷ In addition, mouse SR-BI was unable to bind E2.

Is SR-BI a better candidate receptor than CD81 or the LDL receptor? Identifying a virus receptor or coreceptor is not an easy task because one has to prove that a molecule identified as a putative receptor is indeed necessary to lead to viral infection. Binding to a cell surface molecule is not the only critical step for virus entry, the virus also needs to cross the host cell membrane to deliver its genetic material. For an enveloped virus, the envelope protein(s) play a major role in the entry process by inducing fusion between the viral envelope and the host cell membrane. In the case of HCV, it is very difficult to prove that a candidate molecule is a true receptor. Indeed, because of the lack of an efficient cell culture system to study entry, the classical approach of transfecting a nonpermissive cell line with a cDNA encoding a putative receptor molecule to confer susceptibility to infection is not currently possible for this virus.

In this context, we can only speculate with the information available on the candidate receptors. The LDL receptor and CD81 have a broad tissue distribution, whereas SR-BI is a receptor highly expressed in hepatocytes and steroidogenic tissues. This argument would be in favor of SR-BI as a better candidate. However, receptor expression is not necessarily the sole determinant for viral infection. Other tissue-specific cellular factors might indeed be required for a complete replication cycle.

Because the flaviviruses, which belong to the same family as HCV, are known to enter host cells by endocytosis, it is also believed that an HCV receptor should be able to internalize HCV virions. The LDL receptor has been shown to internalize HCV particles,² and SR-BI internalizes its natural ligand,⁸ suggesting that it might also potentially internalize HCV. In contrast to these two molecules, CD81 has a poor capacity to mediate internalization.⁹ However, it has not been shown that HCV enters the cell by the same route as the flaviviruses, and HCV entry without internalization cannot be totally excluded. Alternatively, HCV binding to CD81 might trigger internalization.

An argument taken by Scarselli et al. in favor of SR-BI and against CD81 is the observation that SR-BI can bind E2 from either genotype 1a or 1b, whereas CD81 does not seem to efficiently recognize E2 of genotype 1b. This is in contrast to another study, however, that has shown that CD81 can interact with E2 from genotypes 1a, 1b, 2a, and 2b.¹⁰

A final argument potentially in favor of SR-BI is the observation that the hypervariable region 1 (HVR1) of E2

is essential for E2-SR-BI interaction. This region is indeed known to contain neutralizing epitopes.¹¹ Despite a strong amino acid sequence variability, the chemophysical properties and the conformation of HVR1 have been shown to be well conserved, in agreement with a potential role in virus attachment.¹² However, it has been reported that an HCV clone lacking HVR1 was still infectious in chimpanzee,¹³ indicating that HVR1 is not essential for infection in this species. In the hypothesis that SR-BI is a receptor for HCV, these data can only be explained by the existence of an alternative receptor.

With several candidate receptors already identified and the current limitations to analyze their role in HCV entry, it is getting more and more difficult to propose a convincing model for HCV entry. In addition, as the search for key molecules potentially involved in HCV entry continues, additional putative receptors will likely emerge, making the situation even more complicated. For this reason, surrogate models to study HCV entry are being developed to go further in studying HCV entry and try to determine the role of candidate receptors.

Virus-like particles have been obtained by expressing HCV structural proteins in insect cells¹⁴ or by reconstituting purified HCV envelope proteins into liposomes.¹⁵ However, these particles are not infectious, and this limits their use mainly to binding studies. Vesicular stomatitis virus pseudoparticles containing modified HCV envelope proteins have also been produced,¹⁶⁻¹⁸ but inconsistencies in the results prevent their use in functional investigations of HCV cell entry.¹⁶ A cell-cell fusion assay has also been developed to study HCV glycoprotein-mediated membrane fusion.¹⁹ However, the transmembrane domains of the envelope glycoproteins used in this assay have been modified to allow their expression at the cell surface. Such modifications lead to the expression of chimeric proteins that have lost their properties of heterodimerization,¹⁸ raising some doubts on the relevance of this approach to study HCV entry.

Very recently, infectious pseudoparticles that are assembled by displaying unmodified HCV glycoproteins onto retroviral core particles have successfully been generated.²⁰ Such particles mimic the early steps of HCV infection and will be very useful to further investigate the role of SR-BI as well as the other currently known and yet to be discovered candidate receptors in HCV entry.

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