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(54) Title: COMPOSITION AND METHOD FOR CONTROLLING HEPATITIS C VIRUS INFECTION

(57) Abstract: Disclosed herein are methods and compositions for the treatment and prevention of Hepatitis C Virus (HCV) infection and methods of screening for antiviral agents against HCV infection and/or production. A method of using compositions of certain apolipoprotein-specific monoclonal or polyclonal antibodies to inhibit HCV infectivity is disclosed. Further, methods of using small interfering RNAs (siRNAs) specific to apolipoproteins for treating and/or preventing HCV infection are disclosed. Also disclosed are methods of using siRNAs specific and/or small molecule inhibitors to certain lipoprotein biosynthetic genes and of using recombinant apolipoprotein E and/or their forms of lipoproteins to treat and/or prevent HCV infections. Screening methods for anti-HCV agents include assessing the effect of a candidate agent on apolipoprotein E and/or apolipoprotein C-I gene expression, assembly, and/or secretion and assessing the effect of a candidate agent on the blockage of the interaction and/or incorporation of HCV nonstructural proteins and/or their fusion forms with reporter proteins into HCV virions.

COMPOSITION AND METHOD FOR CONTROLLING HEPATITIS C VIRUS INFECTION

This application claims the benefit of provisional application no. 60/822,354 titled "Composition And Methods For Treating And Preventing Hepatitis C Virus Infection and Screening Methods For Identifying Anti-Hepatitis C Virus Agents" filed Aug. 14, 2006.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

Research for this invention was made with support from the NCI, Grant Number CA093712, and the NIAID, Grant Number AI51592.

FIELD OF THE INVENTION

The present invention relates to the treatment and prevention of Hepatitis C Virus (HCV) infection and screening for antiviral agents against HCV infection and/or production.

BACKGROUND OF THE INVENTION

The hepatitis C virus (HCV) was discovered in 1989 by molecular cloning and has since been recognized as a major cause of viral hepatitis in humans. HCV is a single-stranded positive-sense RNA virus, which is about 9.6 kb in length. HCV belongs to the *Hepacivirus* genus of the family *Flaviviridae*. The viral RNA genome consists of the 5' untranslated region (5'UTR), a single open reading frame (ORF) encoding a viral polypeptide of 3,010-3,040 amino acids, and the 3' untranslated region (3'UTR) of variable length. Upon translation, the viral polyprotein is cleaved by cellular peptidases and viral proteases into core (C), envelope glycoproteins (E1 and E2), P7, non-structural (NS) proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B. Sequence analysis and comparison studies have revealed that both the 5'UTR and 3'UTR of the HCV genome are highly conserved. In contrast, sequences of the ORF exhibit a variation among HCV isolates. Based on the nucleotide sequence similarity, HCV has been further grouped into six major genotypes and numerous subtypes.

HCV infection is characterized by the establishment of chronic infection in up to about 85% of individuals exposed to HCV. The chronic HCV infection carries an increased risk of developing fatal liver diseases such as cirrhosis, liver failure, and hepatocellular carcinoma.

HCV-associated end-stage liver disease is the leading cause of liver transplantation in the United States (US). It is estimated that approximately 4 million people in the US and 170 million people worldwide are persistently infected with HCV. Each year, HCV infection results in 8,000-10,000 deaths in the US alone. HCV-related deaths are expected to triple within the next 10-20 years if no effective intervention is made available. Currently, there is no specific and effective therapy to treat HCV infection. Accordingly, there remains an urgent need in the art for specific antiviral targets and agents for effectively treating and preventing HCV infection.

The structure and biochemical compositions of HCV virions have not been determined, although certain studies have found that HCV virions isolated from the plasma of hepatitis C patients were associated with lipoproteins to form lipoviroparticles (LVPs). Apolipoproteins B and E were detected in the low-density fractions of HCV RNA-containing particles, which could also be captured by apolipoprotein-specific antibodies, suggesting an association of the low-density HCV virions with human lipoproteins. However, the roles of apolipoproteins in HCV assembly and production have not been defined.

SUMMARY OF THE INVENTION

The present invention addresses the above identified problems, and others, by providing compositions and methods for treating and/or preventing hepatitis C virus infection in humans. The present invention further includes targets and methods for identification (screening) of effective anti-HCV agents.

The present invention discloses a method of using compositions of apoE- and/or apoC-I-specific monoclonal or polyclonal antibodies to inhibit HCV infectivity. The method further comprises the step of administering an effective amount of the composition to a patient.

The present invention discloses methods of using siRNAs specific to apolipoproteins, for treating and/or preventing HCV infection. The present invention further includes siRNAs specific for certain lipoprotein biosynthetic genes for treating and/or preventing HCV infection.

Also disclosed is a method of using recombinant apoE (E2, E3, and E4) protein and/or their forms of lipoproteins to treat and/or prevent HCV infections.

The present invention further discloses a method of screening for anti-HCV agents by assessing the effect of a candidate agent on apoE and/or apoC-I gene expression, assembly, and/or secretion. The present invention also includes a method of screening for anti-HCV agents by assessing the effect of a candidate agent on the blockage of the interaction and/or incorporation of HCV nonstructural proteins and/or their fusion forms with reporter proteins into HCV virions.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the embodiments, is better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, exemplary illustrations of the invention are shown in the drawings. However, the invention is not limited to the specific methods disclosed herein.

FIGURE 1 is a flow chart illustrating an exemplary method for preventing and/or treating Hepatitis C virus (HCV) infection in accordance with the present invention.

FIGURE 2 is a flow chart illustrating an exemplary method of screening for anti-HCV agents in accordance with the present invention.

FIGURE 3A depicts the results of HCV virion RNA (vRNA) determined by a ribonuclease (RNase) protection assay (RPA) in a sucrose gradient sedimentation analysis of HCV virions in culture media and densities (g/ml) of each fraction.

FIGURE 3B is a Western blot analysis of HCV NS3 protein in cells infected with the different fractions identified in **FIGURE 3A**.

FIGURE 3C depicts the results of RPA used to determine positive-strand HCV RNA in cells infected with the different fractions identified in **FIGURE 3A**.

FIGURE 3D is a Western blot analysis of apolipoproteins B100 (apoB100), C-I (apoC-I), and E (apoE), wherein a density gradient sedimentation analysis of HCV RNA-containing particles is performed as shown in **FIGURE 3A**, and apoB100, apoC-I, and apoE proteins were detected by using apoB-, apoCI- and apoE-specific antibodies.

FIGURE 4A depicts the results of a study carried out to determine the HCV-neutralizing activity of apoE-specific monoclonal antibodies, where HCV positive-strand RNA levels in the Huh7.5 cells infected with HCV in the presence of apoE-specific monoclonal antibodies were determined by RPA.

FIGURE 4B is a graph showing the correlation between HCV-neutralizing activity and concentrations of apoE-specific monoclonal antibodies using the quantitative data derived from **FIGURE 4A**.

FIGURE 4C shows the reduction of infectious HCV titers in the cell culture supernatant of Huh7.5 cells that were infected with HCV in the presence of apoE-specific monoclonal antibodies. The infectious HCV titers were determined by immunofluorescence assay (IFA) as foci-forming units per milliliter (ffu/ml) of cell culture supernatant.

FIGURE 4D depicts the results of an analysis of the inhibition of HCV infectivity in the infectious fractions 3 to 5 of the sedimentation analysis as shown in **FIGURES 3B and 3C** by HCV E2- and ApoE-specific monoclonal antibodies.

FIGURE 5 shows the reduction of HCV infectivity by an apoC-I polyclonal antibody, as determined by Western blot analysis of HCV NS3 protein in cells infected with HCV in the presence of increasing concentrations of apoC-I polyclonal antibody.

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