MOLECULAR INSIGHTS INTO THE VIRAL ETIOLOGIES IN LIVER CANCER

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ABSTRACT: Hepatitis B (HBV) and Hepatitis C (HCV) viruses are the main causes of viral hepatitis, increasing the incidence of liver cancer worldwide, especially hepatocellular carcinoma (HCC). The infections caused by HBV and HCV can progress from acute to chronic stages, with an increased risk for HCC due to cirrhosis. Therefore, it is essential to understand their molecular mechanisms of attachment and entry into human cells for developing targeted therapies and preventive measures. This study identifies and analyzes the functional receptors involved in HBV and HCV attachment. It also assesses the expression of genes encoding these receptors in humans and potential animal models. Additionally, the study explores the evolutionary relationship between HBV/HCV and novel HBV/HCV-like viruses identified in various animals. The findings suggest that while HBV and the Capuchin Monkey Hepatitis B virus share common ancestors, the evolutionary relationships between HCV and its animal homologs are less clear, necessitating further investigation.

Keywords: Viral Hepatitis, HBV, HCV, Hepatocellular Carcinoma (HCC), Receptors.

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INTRODUCTION

Viral hepatitis (Hepatitis B and C) causes infection that is divided into two stages; acute and chronic. Acute infection is an illness that is not serious while chronic infection is a serious and lifelong condition [1, 2]. The chronic carriers of both viruses have an increased risk of developing HCC as these viruses promote cirrhosis which is found in 80-90% of HCC patients [3]. Even though both HBV and HCV share similar factors and hosts still they have differences [4].

To develop new therapeutic techniques, it is necessary to understand the role of viral hepatitis in developing liver cancer. After acute infection from the hepatitis B virus, some adults develop subclinical infection in which viral antibodies (one or more) begin to appear leading to a strong response from T helper type 1 (Th1) and cytotoxic T lymphocyte (CTL) cells which results in recovery from infection [5]. However, some patients with acute infection begin to develop chronic infection due to weak Th1 and CTL responses. Most of the carriers of chronic infection do not have any symptoms for decades and they are at higher risk of developing liver diseases, cirrhosis, and HCC. Within a few years, patients with liver disease develop HCC or end-stage liver disease while some recover at an early stage after diagnosis [6]. Similarly, some patients with acute Hepatitis C infection develop strong T helper type 1 (Th1) and cytotoxic T lymphocyte (CTL) responses. This strong response results in their recovery. However, in some patients with acute Hepatitis C infection, the response of cytotoxic T lymphocyte (CTL) and T helper type 1 (Th1) cells is weak [7, 8]. As a result of this weak response, patients begin to develop chronic infections. It is because when cell-mediated immune response is weak, it will trigger and favor the formation of viral mutants that would persist. The chronic infection will then start damaging the liver. The increased damage to the liver yields a strong immune response which leads to the development of a condition called cirrhosis. The cirrhotic condition will result in end-stage liver disease or primary liver cancer called hepatocellular carcinoma (HCC) [9].

Currently, there is no effective vaccine for HCV and treatment is based on medicines including pegylated-interferon-α (IFNα) and ribavirin. The treatment outcome is further improved with the development of DAAs (direct-acting antivirals) [10, 11]. Despite these treatments, HCV infection persists in various regions which might be due to high treatment costs. It justifies an immediate need to develop an effective HCV vaccine that will protect individuals from HCV infection globally. The biggest challenge in designing an HCV vaccine is the variability of virus and sequence diversity [12, 13]. Even though a vaccination to prevent HBV infection has been available for more than thirty years, there are still many people who have a chronic infection. These individuals are susceptible to cirrhosis and hepatocellular carcinoma (HCC) problems. The current course of treatment is effective in preventing the spread of the virus and mitigating the side effects of chronic HBV infection, but it is not curative. Thus, there is a need for novel, finite therapy that can cure chronic HBV infection [14, 15]. Therefore, it is essential to study this virus on a molecular level. It would help in understanding the diversity of the

Hepatitis C virus and designing the HCV vaccine. This study aims to study both Hepatitis B and C viruses at the molecular level. It focuses on receptors for attachment of both viruses in humans and novel HBV/HCV-like viruses identified in other animals.

MATERIALS AND METHODS

Identification of Receptors for Viral Attachment in Human: To design any therapeutic strategy against HBV and/or HCV, it is necessary to understand the attachment of these viruses in humans. The functional receptors for attachment of these viruses in the human body were identified through literature.

Expression Level Analysis of Genes Encoding Proposed Receptors: The receptors for attachment of viral hepatitis are encoded by different genes. The expression of these genes in different organisms will help in understanding the specificity of the host. This information is important in designing animal models for studying the illness of humans. These animal models also help researchers in developing treatments using new drugs. UniProtKB also provided information about the genes that encode these receptors. The expression levels of all the genes coding for proposed receptors needed for viral attachment were analyzed in humans and animals (that are commonly used for animal models like rats, mice, chimpanzees, and tupaia (tree shrew)) by UCSC Genome Browser [17]. UCSC Genome Browser is an open source that graphically displays genome sequences as well as their annotation. It is used to explore genome assemblies.

Identification of Novel HBV/HCV-like Viruses in Animals: The natural hosts of both HBV and HCV viruses are humans. These viruses are also susceptible to chimpanzees [18, 19]. This is because 96% DNA of humans and chimpanzees is identical [20]. There is a lack of reliable animal models for HBV/HCV viruses which is providing hindrance in studying their pathogenicity, virulence, and transmission. It led researchers to search for homologous viruses in different animals. In this step, different HBV/HCV-like viruses were identified in other organisms through literature.

Evolutionary Relationship between HBV/HCV and Novel HBV/HCV-Like Viruses: It is important to determine the source and origin of Hepatitis B and C viruses to prevent their transmission. The evolutionary history and origin of Hepatitis viruses is still unknown and unclear. The genomic sequences of identified Novel HBV and HCV-like viruses were retrieved from the NCBI database [21]. The National Center for Biotechnology Information (NCBI) database contains genomic and biomedical information that is freely accessible to all. The multiple sequence alignment of all these sequences was performed to find their origin using Clustal Omega [22]. The ultimate goal of sequence alignment is the identification of similarities between them. It determines the extent to which these sequences are related which helps in finding evolutionary relation between them

RESULTS

Identified Receptors for HBV/HCV Attachment in Human: Finding receptors of viral attachment in the human body is crucial for understanding how viruses invade host cells and initiate infection. These receptors, often specific proteins on the surface of human cells, serve as gateways for viruses to enter and hijack cellular machinery. Table 1 shows the receptors that have been identified for HBV/HCV attachment in Humans.

Table 1: Receptors identified for HBV and HCV attachments in Humans

Receptor Name	Viral Hepatitis	Reference
Sodium Taurocholate Cotransporting Polypeptide (NTCP)	Hepatitis B	[23, 24]
Heparin Sulfate Proteoglycans (HSPGs	Hepatitis B & C Viruses	[25, 26]
CD81 receptor	Hepatitis C Virus	[27]
Scavenger receptor class B type I (SR-BI)	Hepatitis C Virus	[28]
Mannose-binding lectins DC-SIGN and L-SIGN receptor	Hepatitis C Virus	[29]
Transferin receptor 1	Hepatitis C Virus	[30, 31]
Low-density lipoprotein receptor	Hepatitis B & C Viruses	[32, 33]
The asialoglycoprotein receptor	Hepatitis B & C Viruses	[34, 35]
Tight junction proteins; Occludin (OCLN) and Claudin-1 (CLDN1)	Hepatitis C Virus	[36]

It has been analyzed that the hepatitis D virus requires the Hepatitis B virus for its growth and replication. Therefore, the NTCP receptor is a functional receptor for both HBV and HDV [37].

Expression Level Analysis of Genes Encoding Proposed Receptors: Table 2 gives the expression of genes that encode proposed viral receptors in different organisms. In humans, the gene expression only in liver

cells is provided. It indicates that the genes encoding receptors for viruses' attachment are expressed in humans only. Therefore, the natural hosts of Hepatitis B and C viruses are human and they are incapable of penetrating non-human cells. Chimpanzees were previously preferred in the studies that were designed for preventing and treating human diseases because the genomic makeup of chimpanzees is similar to humans.

Table 0: Expression Level of Genes Encoding Viral Receptors in Liver in Different Organisms

Receptors	Genes encoding receptor	Expression in organisms (RPKM)				
		Human	Tupaia	Mouse	Rat	Chimpanzee
NTCP receptor	SLC10A1	-Liver (62.3)	No Result	No Expression	No Expression	No Result
Heparan sulphate proteo-glycans Receptor	HSPG2	-Liver (1.7)	No Result	No Expression	No Result	No Result
CD81 receptor	cd81	-Liver (176.8)	No Result	No Expression	No Expression	No Expression
Scavenger receptor class B type 1	SCARB1	-Liver (52.3)	No Result	No Expression	No Expression	No Result
Low Density Lipo-protein Receptor	LDLR	-Liver (5.5)	No Result	No Expression	No Expression	No Result
Mannose binding lectins DC-SIGN	CD209	-Liver (0.3)	No Result	No Expression	No Expression	No Expression
Asialogly-coprotein receptor 1	ASGR1	-Liver (248.3)	No Result	No Expression	No Expression	No Result
Asialogly-coprotein receptor 2	ASGR2	-Liver (232.2)	No Result	No Expression	No Expression	No Result
Occludin	OCLN	-Liver (5.0)	No Result	No Expression	No Expression	No Result
Claudin 1	CLDN1	-Liver (55.0)	No Result	No Expression	No Expression	No Result
Transferrin receptor 1	TFRC	-Liver (7.0)	No Result	No Expression	No Expression	No Result

Identified novel HBV/HCV like Viruses: A novel hepatitis B-like virus was identified in Capuchin Monkey which is called Capuchin monkey hepatitis B virus [CMHBV] [38]. The novel Hepatitis C like viruses have been found in rodents [39], dogs (Canine Hepacivirus) [40], cows (Bovine Hepacivirus) [41], and horses (Non-Primate Hepacivirus) [42]. The only hosts that are reported for nonprimate HCV homologs are horses.

Evolutionary Relationship between HBV and Novel HBV-Like Virus: For sequence alignment, the genome, core protein, and X protein sequences of HBV (genotype H) Capuchin Monkey Hepatitis B virus (CMHBV) were obtained. The results of Alignment are shown in Figures 1 & 2.

The results shown in Figures 1 and 2 reveals that there is a high similarity between the genome and core protein sequences of the hepatitis B virus and the Capuchin Monkey Hepatitis B virus. It means there are chances that the actual source of this human-specific virus can be a Capuchin Monkey.

Evolutionary Relationship between HCV and Novel HCV-Like Viruses: For Multiple sequence alignment, the sequences of Canine Hepacivirus (partial), Rodent Hepacivirus, and Bovine Hepacivirus were retrieved. Since the core protein of HCV causes pathogenicity therefore core protein sequences of HCV (genotype 1) were retrieved. The sequence of the core protein of Non-Primate Hepacivirus was not available. The results of Multiple Sequence Alignment (MSA) of the genome and core protein of HCV and novel HCV-like viruses are shown in Figures 3 & 4.

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CapuchinMonkeyHepatitisB    ACCATATCGTCTCTCTTCGCGACAACTGGGGACCCTGTGGAGAACATGGAGAACATCACA  180
HepatitisB(genotypeH)       CTCACATCATCAATCTTCTCGAAGACTGGGGACCCTGCTATGAACATGGAGAACATCACA  169
                              ** *** **  ***** ***  **************    ********************

CapuchinMonkeyHepatitisB    TCAGGATTCCTAGGACCCCTGCTCGCATTACAGGCGGTGTTTTTCTTGTTGACAAAAATC  240
HepatitisB(genotypeH)       TCAGGACTCCTAGGACCCCTTCTCGTGTTACAGGCGGTGTGTTTCTTGTTGACAAAAATC  229
                            ****** ************** ****  ************** ********************

CapuchinMonkeyHepatitisB    CTCACAATTCCTCAGAGTCTCGACTCGTTGTGGACTTCTCTCAATTTTCTAGGGGAGACA  300
HepatitisB(genotypeH)       CTCACAATACCAAAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGTACCA  289
                            ******** **  ******* ******* *************************   **

CapuchinMonkeyHepatitisB    CCCGCGTGTCCTGGCCGAAATTTGCAGTCCCCAACTTGCAGTCACTCACCAACCTCCTGT  360
HepatitisB(genotypeH)       CCCGGGTGTCCTGGCCAAAATTCGCAGTCCCCAATCTCCAATCACTTACCAACCTCCTGT  349
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Figure 1: Similarities between Genome Sequences of HBV and Capuchin Monkey Hepatitis B virus

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CapuchinMonkeyHepatitisB    MHLFHLCLIILCSCPTVQASKQCLGWLLGMDIDPYKEFGATVELLSFLPSDFFPSVRDLL  60
HepatitisB                  MQLFHLCLIISCTCPTVQASKLCLGWLWGMDIDPYKEFGATVELLSFLPSDFFPSVRDLL  60
                            *:******** *:******** ***** **********************************

CapuchinMonkeyHepatitisB    DTASALYREALESPEHCSPHHTALRQTVLCWGELMALASWVGSNLEHPASRELVVNYVND  120
HepatitisB                  DTASALYREALESPEHCSPHHTALRQAILCWGELMTLATWVGNNLEDPASRDLVVNYVNN  120
                            ****************************::*******:**:***.***.****:********:

CapuchinMonkeyHepatitisB    NMGLKIRQLLWFHVSCLTFGRETVLEYLVSFGVWIRTPPAYRPLNAPILSTLPETTVVRR  180
HepatitisB                  NMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRR  180
                            **************:****************************** ****************

CapuchinMonkeyHepatitisB    RP--GSSRGRTPSPRRRRSQSPRRRRSQSPASSC        212
HepatitisB                  RDRGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC        214
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Figure 0: Similarities between Core Protein Sequences of HBV and Capuchin Monkey Hepatitis B virus

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HepatitisCVirus             CCTCGTAATACTCAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTGTCCTTCCTCGT 2650
NonPrimateHepacivirus       TACTATGCGATTGATCGCACTCTCCTACATTGCTGATGATTCTTTGCTGTGGGCTCTGGT 2633
CanineHepacivirus           TACTATGCGATTGATCGCACTCTCCTACATTGCTGATGATTCTTTGCTGTGGGCTCTGGT 2615
RodentHepacivirus           CGAGGTAGTGGCAGCAACAGCCGCCGCTCCTTGGGAGGATATTGTAATGCGGGCCATAGT 2369
BovineHepacivirus           ATGGGTCACCGCTGTTGCCATCGCTTCAACTTGGGATGAATGGTATCTCAGGCTCCTCAT 2203
                                 *          *     *              *       *       *  *

HepatitisCVirus             GTTCTTCTGCTTTGCGTGGTATCTGAAGGGTAGGTGGGTGCCCGGAGCGGTCTACGCCTT 2710
NonPrimateHepacivirus       TTTTTACTGTGTAATCTACTTCACCCCAAGCAGGGTCCCGCCCTTTTGTGTGTTTGTATA 2693
CanineHepacivirus           TTTTTACTGTGTAATCTACTTCACCCCAAGCAGGGTCCCGCCCTTTTGTGTGTTTGTATA 2675
RodentHepacivirus           CTA-CTGTGCTACCCTA------AA--GTACA----------GGTTGCTATCAGTGCCCT 2410
BovineHepacivirus           ATATCTCTTTTTGTGTA------AG--AGTCGCAGGCCTTACCGTTTTATGCTTTGCAAT 2255
                              *     *                                              *
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Figure 1: Similarities between Genome Sequences of HCV and novel HCV-like viruses

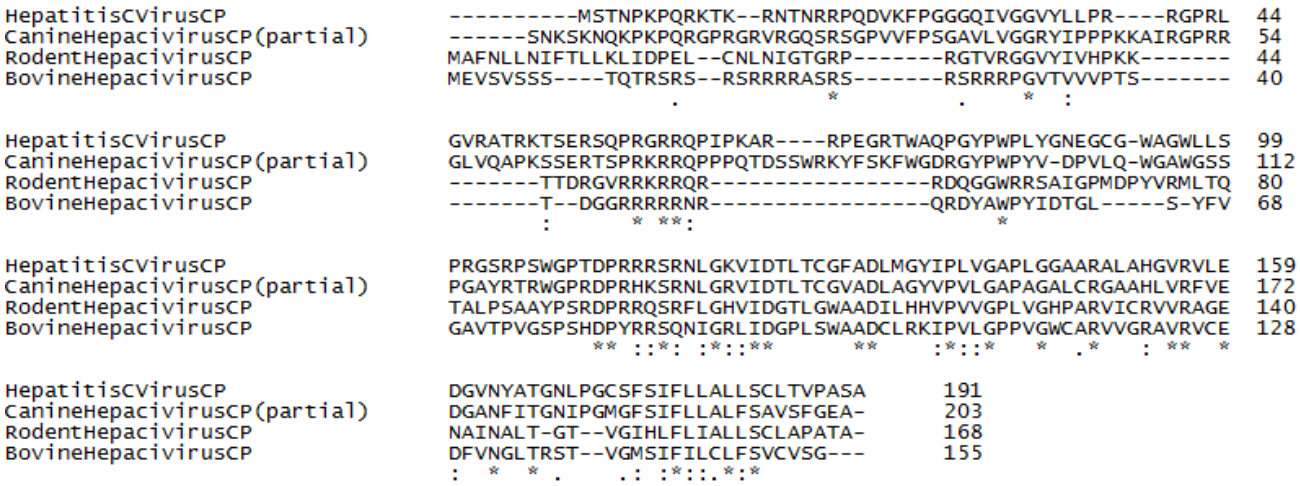

Figure 2: Similarities between Core Protein Sequences of HCV and novel HCV-like viruses

The above results shown in Figures 3 and 4 reveal that there is less similarity between genome and core protein sequences of HCV and novel HCV-like viruses. It means these sequences do not share common ancestors.

DISCUSSION

Hepatitis B and C viruses are serious health problems with high global incidence. They are the leading cause of HCC worldwide. It has been generally agreed that the incidences of HCC will be reduced when there is the active treatment of infections from both of these viruses. Identifying these receptors aids in elucidating the mechanisms of viral pathogenesis, enabling the development of targeted antiviral therapies and vaccines. Furthermore, it provides insights into potential preventive measures and diagnostic tools, enhancing our ability to combat viral diseases and manage outbreaks effectively [43].

One of the most common strategies that can be preferred against HBV/HCV infection is gene silencing. In this strategy, the expression of those genes that are coding for receptors needed by HBV/HCV for their attachment would be prevented [44]. As a result of reduced expression, the receptors would not be formed and there would be no binding of HBV/HCV in humans. Thus, it is important to understand the functions of all proposed receptors before suppressing the expression of any gene so that other mechanisms of the human body would not be affected. For selecting an animal model, the physiological and genetic similarities of animals with humans are also considered. Chimpanzees are susceptible to both of these viruses. However, chimpanzees are now considered an endangered species due to various reasons such as illegal hunting, and climate change so they are currently refrained from being used in animal models. Animals that are currently used for animal models for the Hepatitis C virus include mice, rats, and tupaia [45]. Since, the genes for receptors are only expressed in humans i.e. human specific; therefore, they are artificially inserted in selected animals through mutations. It makes the immune response of these animals weak making them susceptible to HBV/HCV [46].

The animal homologs of viruses infecting humans provide new insights into the origin and evolution of hepatitis viruses along with the host range. The identification of novel HBV/HCV viruses suggests that there is a potential for developing new animal models to study HBV/HCV pathogenesis, treatment, and vaccine design. It will also help in exploring new therapeutic and preventive interventions. It shows that the emergence of these viruses might be from these animals as a result of ancestral co-speciation [47]. From the MSA results, it can be predicted that the hepatitis B virus and the Capuchin Monkey Hepatitis B virus share common ancestors. However, the MSA results of HCV with novel HCV-like viruses focus on the need for further analysis of these viruses to understand their origin. The knowledge about origin and source is essential to control the diseases caused by viruses.

Conclusion: Hepatitis B and C viruses are major contributors to liver cancer, with chronic infections significantly increasing the risk of developing HCC. This study has identified key receptors involved in the viral attachment of HBV and HCV, which are crucial for understanding the pathogenesis and developing therapeutic strategies. The functional analysis of these receptors highlights their essential roles in human physiology, indicating the need for careful consideration before employing gene-silencing approaches for viral inhibition. Furthermore, the expression analysis confirms the human-specific nature of these receptors, complicating the development of animal models but emphasizing the potential of using genetically modified

animals for research. The identification of novel HBV/HCV-like viruses in animals opens new avenues for studying the viruses' origins and developing animal models. While the evolutionary analysis suggests a common ancestry for HBV and the Capuchin Monkey Hepatitis B virus, the relationship between HCV and its homologs remains uncertain, warranting further research. These insights are pivotal for designing effective vaccines and treatments, ultimately aiming to reduce the global burden of hepatitis-related liver cancer.

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