Modeling Hepatotropic Viral Infections

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The hepatotropic viruses A, B, C, D, and E, are the most common causes of viral infections that can lead to liver failure. Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV) are normally transmitted through organ transplants, transfusions, sex, and injections. The HBV genome is a double-strand DNA, and this virus is classified into the genus of Orthohepadnavirus and the family of Hepadnaviridae.

Keywords: animal models ; hepatotropic virus ; cell culture ; viral hepatitis ; modeling hepatotropic viruses

1. Overview

The lack of an appropriate platform for a better understanding of the molecular basis of hepatitis viruses and the absence of reliable models to identify novel therapeutic agents for a targeted treatment are the two major obstacles for launching efficient clinical protocols in different types of viral hepatitis. Viruses are obligate intracellular parasites, and the development of model systems for efficient viral replication is necessary for basic and applied studies. Viral hepatitis is a major health issue and a leading cause of morbidity and mortality. Despite the extensive efforts that have been made on fundamental and translational research, traditional models are not effective in representing this viral infection in a laboratory. In this review, we discuss in vitro cell-based models and in vivo animal models, with their strengths and weaknesses. In addition, the most important findings that have been retrieved from each model are described.

2. Hepatitis Viruses

The lack of an appropriate platform for a better understanding of the molecular basis and the absence of reliable models to identify novel therapeutic agents for a targeted treatment are the two major obstacles for launching efficient clinical protocols in different types of viral hepatitis [1][2]. The hepatotropic viruses A, B, C, D, and E, are the most common causes of viral infections that can lead to liver failure. Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV) are normally transmitted through organ transplants, transfusions, sex, and injections [3]. The HBV genome is a double-strand DNA, and this virus is classified into the genus of *Orthohepadnavirus* and the family of *Hepadnaviridae*. HCV is a small virus (55–65 nm in size) with an RNA genome and is categorized into the genus of *Hepacivirus* and the family of *Flaviviridae* [4]. HDV is a small and uncommon human pathogen containing a single-stranded circular RNA genome. This pathogen is classified into the genus of *Deltaviridae*. HDV has hepatitis B surface antigen (HBsAg), which surrounds the genomic RNA-nucleoprotein complex, thereafter, requires HBV to complete its life cycle and replicate [5][6] HBV, HCV, and HDV induce chronic liver inflammation that can finally result in cirrhosis and hepatocellular carcinoma [5][7]. HAV (Hepatitis A virus) and HEV (Hepatitis E virus) are generally transmitted through water or food [8]. The studies conducted in many countries have demonstrated that HAV is the most common type causing acute viral hepatitis. HAV is a single strand of plus sense RNA and belongs to the genus of *Hepatovirus* in the *Picornaviridae* family, and it is transmitted through the fecal-oral route [9]. HEV is classified into the genus of *Orthohepenvirus A* and the family of *Hepeviridae.* HEV is a nonenveloped positive-strand RNA virus that generally causes acute infections. Clinical observations indicate that HEV can lead to chronic infections in immunocompromised patients, including transplant recipients [8][10]. On the other hand, it is difficult to develop therapeutics for these viral hepatitis, due to the lack of a reliable model. Therefore, establishing a defined and suitable model as a platform for studying hepatotropic viruses is necessary [11]. Studying hepatotropic viruses in vivo is limited because most of them are species-specific. For instance, HBV and HCV infect only humans, tree shrews, and some nonhuman primates [2][12]. Thus, the most promising approach to study human hepatotropic viruses is using genetically modified animals. In parallel, cell-based systems have received remarkable attention in hepatotropic viral infection modeling. Monolayer and three-dimensional (3D) culture systems have been used for understanding the molecular basis of hepatotropic viruses and evaluating novel antiviral agents; however, they did not help the scientific community in understanding some aspects of viral pathogenesis, including the differences between the genotypes of hepatotropic viruses. In this review, we discuss different in vitro and in vivo model systems for the study of hepatotropic viruses, and their advantages and disadvantages.

3. A monolayer Culture (Primary Cells, Cell Lines, and Coculture System)

Primary human hepatocytes (PHH), as the most authentic cell culture model for hepatotropic viruses, were used in many studies [13][14][15]. For in vitro HBV studies, PHH has been used as a gold standard platform in cell culture for many years, and many studies used PHH as a model for HBV and HCV infection [16][17]. A later study indicated that PHH also supported an HEV infection with a Kernow-C1/p6 strain [18]. Other studies demonstrated that PHH was susceptible to HDV [19][20][21]. Taylor et al. provided the first evidence that purinergic receptor functionality was essential for the process of PHH infection by HDV and HBV [22]. Since the source of chronic HEV infection is unknown, some studies explored extrahepatic sources for HEV replication. In 2019, a study [23] introduced human polarized enterocytes (primary intestinal cells) as a model for HEV replication. Moreover, El-Mokhtar and his colleagues demonstrated that primary human endometrial stromal cells were susceptible and permissive to HEV infection, and this type of primary cells could be an endogenous source of HEV infection during pregnancy and mediate the HEV vertical transmission [24]. However, their utility is hampered by some shortcomings, including the viability of donated cells, availability, and limitation for a long-term ex vivo culture [25]. These challenges have led to establishing various culture models [13].

To preserve the liver-specific function and extend the lifetime of PHH, some studies employed a coculture strategy that consisted of PHH and supportive stromal cells (murine fibroblast cells) with the ratio of PHH and supportive cells estimated as 1:4. In this micropatterned coculture system, the phenotype and functionality of PHH were maintained for more than several weeks, and the results showed a robust infection of PHH with HCV [26][27]. Inconsistency with the previous studies, March and collaborators established a micropatterned coculture system in which islands of PHH in a 2D culture were surrounded by fibroblast cells. The results demonstrated that this culture system was suitable for the study of HBV and HCV colonization and replication [28]. Furthermore, Zhou and colleagues applied a coculture system that consisted of fetal PHH and liver nonparenchymal cells for prolonged susceptibility to HBV infection [29]. Winer and collaborators, by self-assembling of PHH and mouse stromal cells in a co-culture system, provided a scalable platform for long-term colonization and replication of HBV [30]. Later, this group demonstrated that self-assembly of PHH with nonparenchymal mouse embryonic fibroblast 3T3-J2 cells in a co-culture system is a versatile platform for studying HBV/HDV coinfections and holds great promise for performing chemical library screens and improving our understanding of the host response to such infections [31]. Furthermore, in 2020, a study [32] reported that peripheral blood mononuclear cells and bone marrow-derived macrophages from healthy donors were susceptible to HEV in vitro. Since renal diseases are associated with HEV infection, in 2020, a study [33] reported a possible mechanism for HEV-mediated renal disease. The authors isolated CD10+/CD13+ primary proximal tubular epithelial cells, infected them in vitro with HEV inoculum, and then the expression of inflammatory and kidney injury markers was assessed in cocultivation with/without immune cells isolated from the same donors. The results demonstrated that coculture of immune cells with HEV-infected epithelial cells exacerbated the inflammatory response and induced kidney injury [33]. However, applying the coculture system for PHH could not overcome the drawbacks of the broad usage of PHH.

Some studies reported establishing two hepatoma cell lines named HepAD38 and HepDE19, which expressed HBV pgRNA under the control of the tetracycline-repressible promoter instead of the native viral core promoter [34][35]. A Chinese group introduced a new hepatoma HLCZ01 cell line which supported the whole life cycle of both HBV and HCV. The results indicated that this cell line provided a powerful tool for supporting the accurate life cycle of the virus with a normal genetic background [36]. Since the HBV infection also occurs at extrahepatic sites, the identification of the relevant host factor in nonhepatic cells is essential. Yang and collaborators reconstituted the HBV infection in the human embryonic kidney (HEK) 293T cells by exogenous expression of the nuclear hormone receptors HNF4α, RXRα, and PPARα, and the HBV receptor, sodium taurocholate cotransporting polypeptide (NTCP). Their results suggested that these factors could play a pivotal role in the HBV infection of nonhepatic cells [37]. A German group established a stable new cell line named HepNB2.7, which was susceptible to HDV and supported the full viral life cycle [38].

Several polarized human liver cell lines were produced, such as HepaRG and HepG2 [39]. The hepatoma HepaRG cell line, a bipotent liver progenitor cell line, upon induction by dimethyl sulfoxide (DMSO), was permissive to HBV [40]. To optimize the in vitro differentiation of HepaRG, Yuan and his colleagues used four small molecules (FPH1, FPH2, FH1, and XMU-MP-1) and increased both the hepatic differentiation and proliferation capacity of HepaRG cells in vitro and in vivo, which is essential for the HBV infection [41]. However, using this cell type has some drawbacks, including a low viral yield and replication, a lack of covalently closed circular DNA (cccDNA) amplification, and difficulty understanding the HBV life cycle [42]. Sophie Roge'e and her colleagues introduced the HepaRG cell line and PICM19, derived from the primary culture of pig embryonic stem cells as in vitro models for HEV replication. They reported that these in vitro culture systems support HEV replication and release of encapsulated RNA [43]. In accordance with this study, Pellerin and her colleagues showed that HepaRG is a relevant and efficient in vitro model of HEV replication that could be used to study HEV and identify effective antiviral drugs against chronic HEV infection [44]. Besides, another study introduced a new procedure

using a cocktail of 5 chemicals (Forskolin, SB431542, IWP2, DAPT, and LDN193189), allowing fast differentiation and efficient HDV-infection of HepaRG cells [45]. Many studies applied the HepaRG cell line as a unique model to study the interplay between HBV/HDV and hepatocyte-specific innate immunity, as well as to explore new therapeutic developments [46][47].

HepG2 [(Hepatoma G2); derived from a hepatoblastoma] and Huh7 [(human hepatocellular carcinoma cell line 7); derived from a hepatocellular carcinoma] are two human hepatoma cell lines which are widely used in antiviral studies, especially those regarding HBV. These cell lines support the virus replication when transfected with HBV [48][49]. Some studies indicated that HepG2 and Huh-7 cell lines as NTCP-expressing lines could be efficiently infected with HBV and HDV [47] [50]. Another study showed altered gene expression in HepG2 cells induced by HBV and HCV, which provides new insight into the mechanism of HBV and HCV infection and improves the understanding of the differences in the molecular pathogenesis of HBV and HCV [51]. Not long ago, it has been found that HepG2-NTCP cells are hardly infected with HBV-positive sera and that a clonal section is needed to recognize clones producing high titers of infectious progeny [52]. Recently, Kempp et al. established a stable cell line (HepNB2.7) by transducing HepG2 cells with genes encoding the NTCP-receptor and the HBV envelope proteins that support the full viral life cycle of HDV and HBV [38]. Furthermore, to support HEV replication, in a study [53] HepG2 and Huh-7 lines were used. A study [54] reported a simple yet robust cell culture HEV infection method. The model was based on the HEV genotype 3 Kernow-C1 p6 strain and the two human hepatoma cell lines (HepG2 and HepG2/C3A) combined with various media conditions.

To investigate various viruses' interactions, Jian and collaborators developed a scalable and visualizable HAV/HCV coinfection model in Huh-7 cells. Their finding revealed that the simultaneous presence of HAV-HCV did not affect the viral RNA synthesis of both viruses. They suggested that indirect interactions may lead to the suppression and clearance of HCV in HAV/HCV coinfected patients [55]. Sun and colleagues reported the creation of stable TetOFF hepatoma cell lines (HepG2 and Huh7) to control HBV production. Their approach presented some advantages, including applying both hepatoma cell lines and using a two-step procedure rather than cotransfection [56]. König and collaborators tried to develop a perfect cell culture platform for HBV amplification from clinical specimens. To achieve this, they applied slow proliferating HepG2- NTCP for the HBV infection. The obtained results demonstrated that this cell line successfully supported the whole HBV life cycle, as well as long-term amplification of HBV [52]. However, these cell lines could do mediate the early-stage virus infection, such as the entry, uncoating, and the formation of cccDNA [25]. Besides, the causes of limited HCV permissiveness in cell lines are not completely understood, but the most important aspects have been identified. Restricted expression of cell surface receptors, such as CD81 and scavenger receptor class B type I (SRBI), is recognized to be associated with the restriction of the HCV entrance [25][57][58].

Therefore, to better mimic the viral life cycle and host-virus interactions, more representative and functional cell types are urgently needed. Pluripotent stem cells (PSCs) are a renewable source of cells and can be obtained via different protocols [59]. Since the cells derived from PSCs would be functional and similar to primary cells, they may be suggested as an ideal replacement for currently used cell-based models [60]. In 2012 and 2015, 2D cultures of HLCs derived from human PSCs were shown to support the entry and replication of HCVcc [61][62]. In 2017, these findings were corroborated by Yan et al. [63]. Various host factors are critical to HBV infection. Hepatic-like cells (HLCs) mostly resemble PHH, due to the high expression of crucial factors for HBV infection and replication. As a first confirmation, Shlomi and colleagues indicated that PSCs-derived HLCs were permissive to the HBV infection [27]. Consistent with the previous study, two other studies demonstrated that stem cell-derived HLCs could completely support the HBV infection for about one month [25][63][64]. Moreover, several studies reported that PSCs-derived HLCs could successfully support various forms of HCV infection [65] [66]. Some studies have successfully demonstrated that the complete replication cycle of HEV is supported by iPSC-derived HLCs [67][68][69]. In cell-based models of liver disease, researchers understand the importance of hepatocyte polarity. Because these viruses enter hepatocytes through the basolateral membrane, the hepatocyte polarity is crucial for the productive entry of hepatitis viruses [70]. In one study [70], researchers differentiated hPSCs into columnar polarized HLCs using transwell filters. These HLCs secreted urea, albumin, and lipoproteins basolaterally, while bile acids were produced apically. The authors showed that polarized HLC supported HEV infection and replication, and mimicked fundamental steps of the natural infectious cycle in vivo.

Nevertheless, although great efforts are directed toward improving differentiation protocols to achieve better maturation, little progress has been achieved so far. Therefore, there is a need to provide a suitable niche that is more similar to the in vivo architecture, such as spheroids, organoids, bioprinted microtissues, and microfluidic chip devices [60].

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