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Pan-Genotype Hepatitis E Virus Replication in Stem Cell–Derived Hepatocellular Systems

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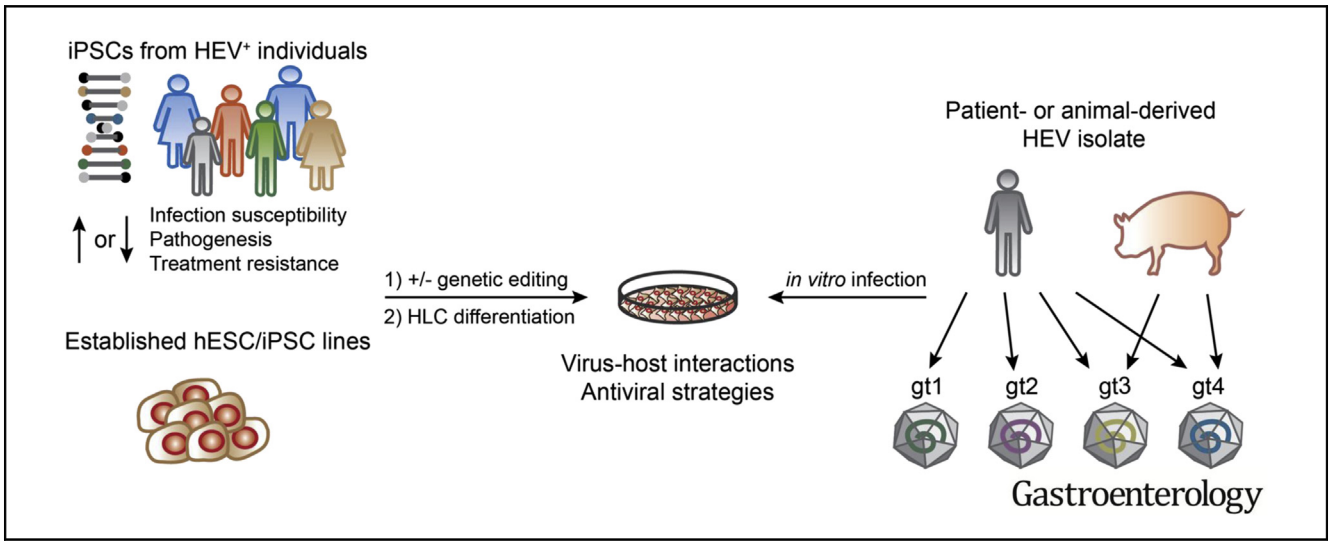

BACKGROUND & AIMS: The 4 genotypes of hepatitis E virus (HEV) that infect humans (genotypes 1–4) vary in geographical distribution, transmission, and pathogenesis. Little is known about the properties of HEV or its hosts that contribute to these variations. Primary isolates grow poorly in cell culture; most studies have relied on variants adapted to cancer cell lines, which likely alter virus biology. We investigated the infection and replication of primary isolates of HEV in hepatocyte-like cells (HLCs) derived from human embryonic and induced pluripotent stem cells. **METHODS:** Using a cell culture–adapted genotype 3 strain and primary isolates of genotypes 1 to 4, we compared viral replication kinetics, sensitivity to drugs, and ability of HEV to activate the innate immune response. We studied HLCs using quantitative reverse-transcriptase polymerase chain reaction and immunofluorescence assay and enzyme-linked immunosorbent assays. We used an embryonic stem cell line that can be induced to express the CRISPR-Cas9 machinery to disrupt the peptidylprolyl isomerase A gene, encoding cyclophilin A (CYPA), a protein reported to inhibit replication of cell culture–adapted HEV. We further modified this line to rescue expression of CYPA before terminal differentiation to HLCs and performed HEV infection studies. **RESULTS:** HLCs were permissive for infection by nonadapted, primary isolates of HEV genotypes 1 to 4. HEV infection of HLCs induced a replication-dependent type III interferon response. Replication of primary HEV isolates, unlike the cell culture–adapted strain, was not affected by disruption of the peptidylprolyl isomerase A gene or exposure to the CYPA inhibitor cyclosporine A. **CONCLUSIONS:** Cell culture adaptations alter the replicative capacities of HEV. HLCs offer an improved, physiologically relevant, and genetically tractable system for studying the replication of primary HEV isolates. HLCs could provide a model to aid development of HEV drugs and a system to guide personalized regimens, especially for patients with chronic hepatitis E who have developed resistance to ribavirin.

EDITOR'S NOTES

BACKGROUND AND CONTEXT

Limited cell culture options restrict studies and thus our knowledge on the biology of the four hepatitis E virus (HEV) genotypes that can infect humans.

NEW FINDINGS

In contrast to hepatoma cells, stem cell-derived hepatocyte-like cells are permissive for infection by non-adapted HEV isolates of all four genotypes.

LIMITATIONS

Future studies with additional isolates are needed to allow definitive comparisons between HEV genotypes.

IMPACT

These results reinforce the importance of using physiologically more relevant culture systems for HEV studies.

Keywords: HLCs; Antiviral; Primary Isolates; Personalized Medicine.

Hepatitis E virus (HEV) is among the most common causes of acute hepatitis in the world.[1] It is a positive-strand RNA virus and a member of the *Orthohepevirus* genus within the Hepeviridae family. Four major genotypes (gt), gt1 to 4, can infect humans. Gt1 and gt2 are restricted to humans and lead to large outbreaks in developing regions. Gt3 and gt4 can be transmitted zoonotically through the ingestion of infected meat and cause infections also in the developed world (reviewed in Meng[2]). A single case study suggests that camelid HEV gt7 can be transmitted to humans.[3] The severity of HEV-associated hepatitis seems to correlate with the status of the host's immune system; but viral factors also may play important roles in the pathogenesis of the disease.[4] Indeed, gt1 and gt2 have vastly different epidemiological patterns from gt3 and gt4. Gt1 and gt2 lead to infections and illness in young healthy individuals, with high fatality rates of pregnant women. Gt3 and gt4 mostly infect the middle-aged and elderly, with men being the most likely to experience severe disease (reviewed in Kmush et al[5]). Altogether, additional studies of HEV genotypes are needed to better understand viral transmission and pathogenesis.

The HEV 7.2-kb positive-strand RNA genome harbors at least 3 open reading frames (ORFs). ORF1 encodes the replicase, ORF2 the capsid protein, and ORF3 a small protein involved in virus secretion (reviewed in Meng[2]). Recently ORF4 has been identified in gt1 isolates, which is necessary for RNA replication under conditions of endoplasmic reticulum stress.[6]

A detailed understanding of HEV molecular biology has been hampered by the absence of efficient cell culture systems. Only recently, major advances were made by passaging primary gt3 and gt4 HEV isolates and selecting variants that replicate in carcinoma cells. These infectious, cell culture–adapted viruses have made the study of the entire HEV life cycle possible. Similar efforts have not been as successful for gt1 or gt2 isolates. Emerson and colleagues[7] developed an HEV gt1 cell culture system that supports the full replication cycle of the Sar55 strain; however, this system produces only low virus titers.

The mutations found in the gt3 and gt4 HEV cell culture–adapted viruses are not limited to single base changes. Some also include insertion of sequences derived from human host genes or the viral genome itself into the hypervariable region of ORF1.[8–10] These insertions appear to confer a replicative advantage in cancer cells as they become the dominant viral species on passaging; however, by doing so they likely alter HEV biology. In this regard, HEV mimics other human hepatotropic viruses, such as hepatitis A virus and hepatitis C virus (HCV), which usually also require adaptive mutations to replicate efficiently in cell culture.[11,12]

Another limitation of current hepatotropic virus cell-culture systems is their heavy reliance on cancer-derived cell lines. These cell lines are typically de-differentiated and have altered metabolic, innate immune, and apoptotic responses. Primary human hepatocytes (PHHs) present an attractive alternative for studying hepatotropic viruses. They are certainly more physiologically relevant. However, PHHs have drawbacks. They are highly variable, expensive, and difficult to maintain and manipulate genetically. There is, however, another alternative: human embryonic (hESCs) and induced pluripotent stem cells (iPSCs). Unlike PHHs, these cells provide a renewable resource and can be genetically manipulated to create patient-specific disease models. Further, they can be differentiated into many cell types, including hepatocytes. We previously found that hESC- or iPSC-derived hepatocyte-like cells (HLCs) are permissive for hepatitis B virus (HBV) and HCV[13–15] infection. We and others[16,17] showed that HLCs are permissive for virus derived from an infectious HEV gt3 Kernow-C1 cDNA clone that was selected after 6 serial passages (P6) in HepG2 cells. Here we show that HLCs, in contrast to hepatoma cells, are not only permissive for the P6 variant but also for virus derived from an early passage (passage 1 [P1]) of the same isolate in which the recombinant genome is only a minor species. Moreover, we found that HLCs are fully permissive for infection and replication of HEV primary isolates of gt1 to 4 derived from infected animals. Thus, for the first time, HLCs allow in vitro studies of nonadapted HEV of all genotypes that infect humans.

***Authors share co-first authorship.**

Abbreviations used in this paper: **ALB, albumin; AFP, alpha-fetoprotein; bFGF, basic fibroblast growth factor; BM, basal medium; CsA, cyclosporine A; DE, definitive endoderm; DMSO, dimethyl sulfoxide; Dox, doxycycline; ESC, embryonic stem cell; gt, genotype; HBV, hepatitis B virus; HCM, hepatocyte culture medium; HCV, hepatitis C virus; HepProg, hepatic progenitor; hESC, human embryonic stem cell; HEV, hepatitis E virus; HEVcc, cell culture grown HEV; HLCs, hepatocyte-like cells; hPSC, human pluripotent stem cells; IC_{50}, inhibitory concentration; IFN, interferon; ImHep, immature hepatocyte; iPSC, induced pluripotent stem cells; ISG, interferon-stimulated gene; KO, knockout; ORF, open reading frame; P1, passage 1; P6, passage 6; PHH, primary human hepatocytes; p.i., postinfection; PPIA, cyclophilin A; RdRp, RNA-dependent RNA polymerase; RBV, ribavirin; SOF, sofosbuvir; WT, wild-type.**

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