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Development of an efficient reverse genetics system for HEV genotype 1 for use in comparative studies with zoonotic genotype 3

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Hepatitis E virus (HEV) genotype 1 (gt1) infects only humans whereas genotype 3 (gt3) is zoonotic infecting various animal species and humans. For elucidation of determinants of host specificity, experiments using reverse genetics enabling targeted genome manipulations are needed. However, whereas a few reverse genetics systems exist for gt3, those for gt1 are still very limited, mainly because of inefficient replication of this genotype in cell cultures. Here, the generation of gt1 strain Sar55 and gt3 strain 47832mc by transfecting in vitro transcribed and capped virus genomes into different cell lines was attempted. Transfection of the human hepatoma cell lines PLC/PRF/5 and HuH7-Lunet-BLR resulted in increasing amounts of viral RNA and protein for both genotypes; however, significant numbers of infectious particles were only present in case of gt3. In contrast, transfecting the intestinal cell line Caco2 resulted in the release of infectious gt1 and, to a lesser extent, gt3 into the supernatant. Gt1 reached titers >1000 infectious particles/ml and could be readily passaged on fresh Caco2 cells. Density gradient analyses indicated that the majority of gt1 virus particles were enveloped in analogy to gt3. In contrast, less secreted capsid protein was present in case of gt1 compared to gt3. The results indicate that gt1 is able to exert a complete replication cycle in Caco2 cells and provide an efficient reverse genetics system for this genotype for future studies.

Keywords

Hepatitis E virus, cell culture, replication, transfection

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Professional Status of the Speaker

Postdoc

Junior Scientist Status

No, I am not a Junior Scientist.

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