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Journal of Hepatology: Journal Club

siRNA – a powerful tool to unravel hepatitis C virus-host interactions within the infectious life cycle

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Hepatitis C virus (HCV) is a major cause of liver disease including liver cirrhosis and hepatocellular carcinoma (1). Current therapies based on pegylated interferon and ribavirin are characterized by limited efficacy, high costs and substantial side effects (1). Moreover, the clinical development of novel antiviral substances targeting HCV protein processing and replication is progressing more slowly than expected due too safety issues and viral resistance (1). Thus, novel antiviral treatment strategies targeting interactions of the virus with the host cells during entry, replication, assembly and export may offer an approach to overcome viral resistance complementing current antiviral therapies. The prerequisite for developing novel antiviral strategies is a detailed understanding of virus-host interactions during HCV infection. During the last two years, the knowledge of HCV-host interactions is rapidly advancing following the establishment of an HCV tissue culture model allowing the study of the entire life cycle (2-4). Using this HCV tissue culture system, Randall and colleagues from Charlie Rice's laboratory at Rockefeller University, New York, demonstrated that gene silencing by siRNA elegantly allows to unravel HCV-interactions during the infectious life cycle (5).

Small or short interfering RNAs (siRNA) mediate the degradation of complementary mRNA that results in a specific silencing of gene expression (6). siRNA mimics cellular microRNA (miRNA) that consists of small double stranded RNA strands and regulates gene expression by degrading the complementary mRNA. A key protein for the generation of these molecules is DICER, an RNase enzyme that is responsible for the production of short interfering RNAs and microRNAs (6). Specific siRNAs targeting the expression of individual cellular cofactors are successfully applied as tools to study the impact of defined host factors for HCV entry (7) and replication (8). Furthermore, functional siRNA screens have been shown to represent a powerful tool to identify novel cellular co-factors for pathogen-host interactions (9).

Aiming to unravel HCV-host interactions during the HCV life cycle, Randall and colleagues first performed a yeast-two-hybrid screen resulting in the identification of 10 cellular proteins interacting with HCV non-structural protein NS5A (5). To confirm the functional impact of the identified host cell molecules for HCV infection by gene silencing, the authors designed specific siRNAs targeting the expression of the identified proteins. In addition, siRNAs were designed for 52 known interaction partners of HCV proteins and members of signaling pathways that are thought to influence HCV replication (5). These included siRNAs targeting the well characterized HCV entry factor CD81 or the dead-box helicase DDX3X to verify the validity of the approach.

Interestingly, the authors observed that silencing of 26 genes modulated virus production more than 3 fold. Examples for cellular proteins with a significant impact on the HCV life cycle identified by Randall et al. (5) are depicted in Fig. 1. Among these hits were eight proteins (including RAF1, VAP-A/B and PTBP1) that impair HCV genotype 1 replication if inhibited or inactivated as reported previously (5). Taken together, this first part of the study identified several novel cellular co-factors required for productive HCV infection and confirmed the functional impact of several candidates for HCV infection previously identified in other surrogate HCV model systems.

In the second part of their study, Randall and colleagues embarked to investigate the impact of the cellular RNA interference (RNAi) machinery for the HCV life cycle. RNA interference has been shown to be a major component of the innate immune response to viral infection in both plants and invertebrate animals (10). However, accumulating evidence suggests that RNAi is not a robust antiviral pathway in mammals (10). If RNAi represents a general antiviral mechanism it is likely that a virus would try to inhibit the RNAi machinery to ensure its own replication as it could be shown for plant viruses (10). Contradicting data on

this issue had been previously published using HCV genotype 1 replicons: Whereas a report from P. Sarnow's lab had shown convincing evidence that the micro RNA miR-122 is required for HCV replication (11), Wang et al. reported that DICER siRNAs -which would interfere with miR-122 synthesis- enhanced HCV replication (12). To address this important question, Randall et al. first silenced multiple components of the RNAi pathway and tested the effects on HCV replication and virus production in the HCV infectious tissue culture system. Interestingly, Randall and colleagues found that an independent silencing of several components of the RNAi machinery including DICER1 markedly reduced HCV production and intracellular HCV RNA levels (5). Furthermore, by depleting miR-122 in Huh7.5 hepatoma target cells, Randall et al. confirmed that the presence of miR-122 is important for HCV replication and virus production. In a third experimental approach, Randall et al. investigated the miRNA environment associated with HCV infection by profiling of the relative miRNA expression of liver, Huh7.5 cells and Huh7.5 cells harboring the HCV Con1 replicon. Interestingly, miR-122 was the most abundant miRNA and its levels were not affected by HCV replication. Thus, the authors conclude that (i) HCV replication does not grossly affect micro RNA biogenesis and (ii) that the requirement for functional RNAi for HCV replication is dominant over any antiviral activity this pathway may exert against HCV. Furthermore, the findings implicate the cellular RNAi machinery as a novel antiviral target to inhibit HCV replication.

What are the perspectives resulting from the study of Randall and colleagues for the understanding of the pathogenesis of HCV infection and the development of novel antivirals? Although the impact of most of the identified host interactions has not yet been confirmed *in vivo*, it is likely that at least some of the findings play an important role for pathogenesis of HCV infection. This hypothesis is supported by the fact that the virus produced in the infectious tissue culture model is infectious in chimpanzees *in vivo* (2, 3) and key findings of

Randall et al. (such as the impact of the RNAi machinery) have been already confirmed in model systems based on other HCV genotypes (5).

Finally, the study of Randall and colleagues elegantly demonstrates the potential of siRNA technology to unravel virus host-interactions within the viral life cycle. siRNA technology in combination with the HCV infectious tissue model conveniently allows to verify the functional impact of candidate host cell factors identified in non-infectious model systems (such as the RNAi machinery (5)). Furthermore, this study highlights the reliability and potential of siRNA technology itself to identify novel interaction partners of HCV and thus paves the way for functional large scale siRNA screens using the HCV tissue culture system. Such a functional screen would doubtless advance the current understanding of the viral life cycle and could identify novel targets for urgently novel antiviral therapies.

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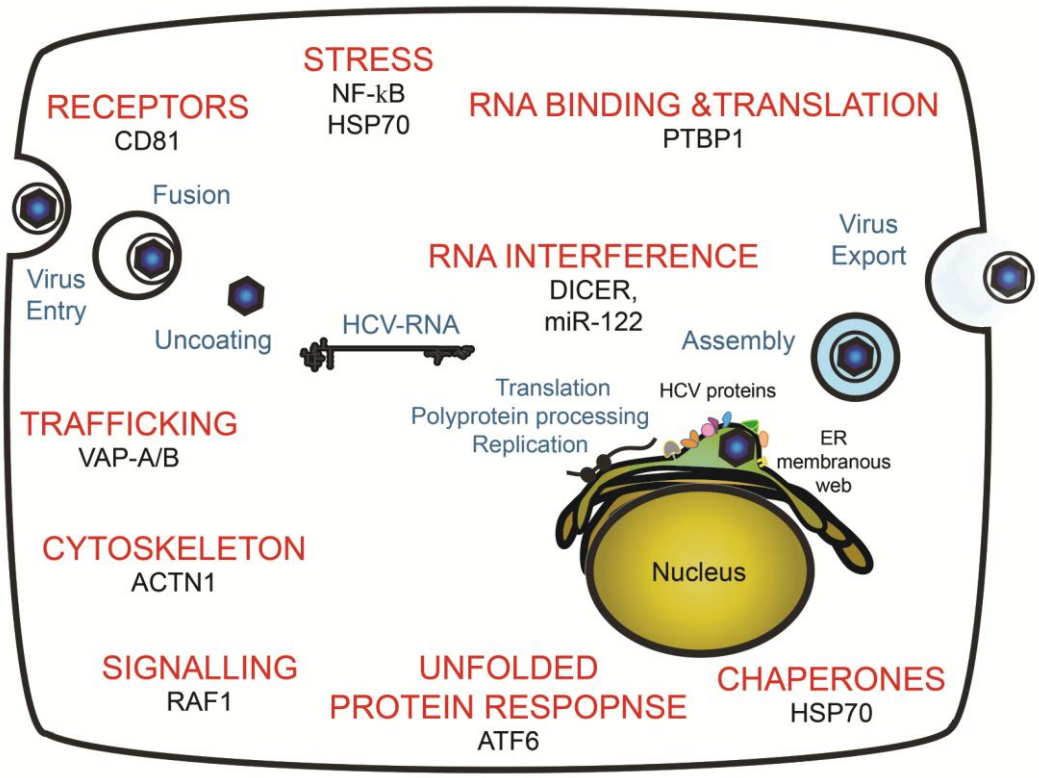
FIGURE LEGEND

Fig. 1. HCV-host interactions with functional impact on the HCV life cycle investigated by Randall et al. using siRNA (5). The putative HCV life cycle including entry, uncoating, translation, polyprotein processing, replication, assembly and export is shown in blue. Examples of cellular co-factors with a functional impact on the HCV infection in the HCV tissue culture system (5) are depicted in relationship to the respective steps of the viral life cycle. Gene abbreviations refer to Human Genome Organization nomenclature (HUGO): NF-kB - nuclear factor of kappa light chain gene enhancer in B-cells, PTBP1 - polypyrimidine tract binding protein 1, miR-122 - micro-RNA type 122, HSP70 - heat shock protein 70 kDa, ATF6 - activating transcription factor 6, RAF1 - v-raf-1 murine leukemia viral oncogene homolog 1, ACTN1 - actinin alpha 1, VAP-A/B - vesicle-associated membrane protein A and B.

FIG. 1.


STRESS
NF-kB
HSP70
RECEPTORS
CD81
RNA BINDING &TRANSLATION
PTBP1
Fusion
Virus
Entry
Uncoating
RNA INTERFERENCE
DICER,
miR-122
HCV-RNA
Virus
Export
Assembly
Translation
Polyprotein processing
Replication
HCV proteins
ER
membranous
web
TRAFFICKING
VAP-A/B
CYTOSKELETON
ACTN1
Nucleus
SIGNALLING
RAF1
UNFOLDED
PROTEIN RESPOPNSE
ATF6
CHAPERONES
HSP70