



HAL
open science

Impact of OGT on late steps of the hepatitis C virus replication cycle

Katharina Herzog

► **To cite this version:**

Katharina Herzog. Impact of OGT on late steps of the hepatitis C virus replication cycle. *Virology*. Université de Strasbourg, 2019. English. NNT : 2019STRAJ085 . tel-02918254

HAL Id: tel-02918254

<https://theses.hal.science/tel-02918254>

Submitted on 20 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ÉCOLE DOCTORALE 414

UMR_S 1110 Institut de Recherche sur les maladies virales et hépatiques

Thèse

présentée par

Katharina HERZOG

soutenue le 29 Octobre 2019

Strasbourg, France

Pour obtenir le grade de : **Docteur de l'Université de Strasbourg**

Discipline/Spécialité : Sciences de la Vie et de la Santé

Aspects moléculaires et cellulaires de la Biologie

Impact of OGT on late steps of the hepatitis C virus replication cycle

THÈSE dirigée par :

Dr. Mirjam B. ZEISEL

Prof. Thomas F. BAUMERT

RAPPORTEURS :

Prof. Philippe ROINGEARD

Dr. Annette MARTIN

AUTRE MEMBRE DU JURY :

Prof. Philippe GEORGEL



UNIVERSITÉ DE STRASBOURG



ÉCOLE DOCTORALE 414

UMR_S 1110 Institut de Recherche sur les maladies virales et hépatiques

Thèse

présentée par

Katharina HERZOG

soutenue le 29 Octobre 2019

Strasbourg, France

Pour obtenir le grade de : **Docteur de l'Université de Strasbourg**

Discipline/Spécialité : Sciences de la Vie et de la Santé

Aspects moléculaires et cellulaires de la Biologie

Impact of OGT on late steps of the hepatitis C virus replication cycle

THÈSE dirigée par :

Dr. Mirjam B. ZEISEL

Prof. Thomas F. BAUMERT

RAPPORTEURS :

Prof. Philippe ROINGEARD

Dr. Annette MARTIN

AUTRE MEMBRE DU JURY :

Prof. Philippe GEORGEL

This thesis was supported by the international PhD program of the University of Strasbourg with an initiative of excellence (IdEX) scholarship.

Success

He has achieved success

Who has lived well, laughed often, and loved much;

*Who has enjoyed the trust of pure women, the respect of intelligent men and
the love of little children;*

Who has filled his niche and accomplished his task;

*Who has left the world better than he found it whether by an improved poppy,
a perfect poem or a rescued soul;*

Who has never lacked appreciation of Earth's beauty or failed to express it;

Who has always looked for the best in others and given them the best he had;

Whose life was an inspiration;

Whose memory a benediction.

[Bessie Anderson Stanley]

ACKNOWLEDGEMENTS

A big THANK YOU to my thesis committee, Prof. Philippe Roingear, Dr. Annette Martin and Prof. Philippe Georgel for taking your limited time to read my thesis and participate in my thesis defense!

I would like to thank, Prof. Thomas Baumert, for giving me the opportunity being a PhD student in your lab and for being my teacher in many situations.

I would like to express my heartfelt thanks to Dr. Mirjam Zeisel. Words cannot express how thankful I am being your PhD student: I have learnt so many things from you from basic technical issues, preparing good presentation to at least scientific writing. You always gave me the feeling that I can ask you all kind of questions and taught me how to handle even difficult situations!

I give my special thanks to Dr. Simonetta Bandiera. Thank you for your help when I arrived in Strasbourg, for your practical tips and your way of explaining even a difficult context!

I express my gratitude to all members of the unit, past and present. Thank you for helping me learning all the experimental skills, for your help in administrative and technical issues, for supporting me during these three years and for always helping me when I had questions or when I needed help!

Mum, du wundervoller Mensch, was haben wir schon alles durchgestanden, ich bin so dankbar dich an meiner Seite zu wissen. Du bist nicht nur meine Mum, sondern viel mehr meine engste Vertraute, meine Freundin und meine erste Anlaufstelle, wenn ich Rat brauche, diejenige, die mich in den Arm nimmt, Trost spendet und mir zur richtigen Zeit einen Schubs in die richtige Richtung gibt. Das war immer so und wird auch immer so bleiben! Danke für deine Unterstützung und deine Liebe.

Dad, du allwissendes Lexikon, so gerne hätte ich manche Fähigkeiten von dir. Du hast mich unterstützt, immer und bedingungslos. In meinen tiefsten Tiefs hast du mit deiner ehrlichen und direkten Art und deiner Fähigkeit, manche Dinge auf ihre Fakten herunterzubrechen, geholfen Dinge aus einer anderen Perspektive zu betrachten, aber genauso hast du dich in meinen höchsten Hochs mit mir gefreut. Weil ich es vielleicht noch nie so gesagt habe: du bist ein unverzichtbarer Bestandteil in meinem Leben!

Philipp, mein kleiner wunderbarer Bruder und *Partner in crime*. Deine Geburt hat nicht nur meine Kindheit, sondern mein ganzes Leben bereichert. Ohne dich, wäre ich nur halb ich! Worte können an dieser Stelle nicht beschreiben, wie wichtig und unverzichtbar du für mich bist!

Last but not least, Manu, *my love*. Danke für dein offenes Ohr, deine tröstenden Worte, deine Freude, deine Fähigkeit selbst in größter Dunkelheit Licht zu sehen, dafür das wir über unsere kleinen Insider so herzlich lachen können und für deine bedingungslose Liebe!

Table of Contents

I.	HEPATITIS C – An Introduction	2
1.1	Pathogenesis of HCV infection	3
1.2	HCV – Basic virology	4
1.2.1	Genomic organization of HCV RNA.....	4
1.2.2	Genetic diversity of HCV – One challenge for vaccine design	5
1.2.3	HCV virion structure: The lipoviral particle.....	5
1.2.4	The HCV life cycle	7
1.3	Model systems to study HCV.....	17
1.3.1	<i>In vitro</i> model systems	17
1.3.2	<i>In vivo</i> model systems	21
1.4	HCV treatment	23
II.	PROTEIN GLYCOSYLATION	28
2.1	Post-translation modifications of HCV proteins	28
2.2	<i>O</i> -linked <i>N</i> -acetylglucosaminylation (<i>O</i> -GlcNAcylation)	31
2.3	OGT and <i>O</i> -GlcNAcylation in disease and cancer	34
III.	OBJECTIVES	38
IV.	RESULTS	40
V.	DISCUSSION	46
VI.	RÉSUMÉ DE LA THÈSE DE DOCTORAT	50
VII.	REFERENCES.....	56
VIII.	ANNEX.....	78
IX.	CURRICULUM VITAE	82

List of Figures

Figure 1. Estimated global HCV prevalence in 2015.	2
Figure 2. Acute (resolving) HCV infection versus chronic HCV infection.....	3
Figure 3. Overview of (A) HCV genome organization and IRES-dependent translation into polyprotein and (B) viral proteins and their function.	4
Figure 4. Infectious LVP versus VLDL.....	7
Figure 5. HCV attachment and entry into hepatocytes.	9
Figure 6. HCV replication and formation of the membranous web.....	12
Figure 7. Potential function of the cytosolic nuclear pore complexes in HCV replication and assembly.....	13
Figure 8. Model of the formation of LVPs and comparison to cell culture-derived HCV (HCVcc).16	
Figure 9 In vitro HCV cell culture models.	19
Figure 10. Host-targeting agents (HTAs) at different steps of the HCV replication cycle.....	26
Figure 11. Basic principle of N- and O-linked glycosylation, and phosphorylation.	29
Figure 12. Schematic representation of N-glycosylation sites of E1 and E2.....	30
Figure 13. O-GlcNAcylation versus phosphorylation.	32
Figure 14. The three isoforms of OGT.	32
Figure 15. Functional distribution of identified O-GlcNAcylated proteins.....	34
Figure 16. miRNAs involved in the HCV life cycle and expression in Huh7.5.1 cells.....	38

List of Tables

Table 1. Combinational DAA treatment.	24
Table 2. O-GlcNAc dynamics in different types of cancer.....	36

ABBREVIATIONS

aa	Amino acids
ApoB	Apolipoprotein B
ApoC	Apolipoprotein C
ApoE	Apolipoprotein E
CD81	Cluster of differentiation 81
CLDN1	Claudin-1
DAA	Direct-acting antiviral
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
gt	Genotype
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCVcc	Cell culture-derived HCV
HCVpp	HCV pseudoparticles
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
Huh7	Human hepatoma 7
INF- α	Interferon-alpha
INF- γ	Interferon-gamma
IRES	Internal ribosome entry site
JFH1	Japanese Fulminant Hepatitis I
kb	Kilobase
kDa	Kilodalton
LD	Lipid droplet
LDL	Low-density lipoprotein
LDLR	Low-density lipoprotein receptor
LPL	Lipoprotein lipase
LVP	Lipoviral particle

miR	micro-RNA
MLV	Murine leukemia virus
mRNA	messenger RNA
MTP	Microsomal triglyceride transfer protein
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
ncRNA	non-coding RNA
OGA	β -N-acetylglucosaminidase
OGT	O-linked N-acetylglucosamine transferase
OCLN	Occludin
PHH	Primary human hepatocytes
RIG-I	Retinoic acid-inducible gene I
RNA	Ribonucleic acid
RNAi	RNA interference
SCID	severe combined immunodeficiency
siRNA	small interfering RNA
SR-BI	Scavenger receptor class B type I
TCID ₅₀	50% tissue culture infectious dose
TJ	Tight junction
TLR	Triglyceride-rich lipoprotein
uPA	urokinase plasminogen activator
UTR	Untranslated region
VLDL	Very low-density lipoprotein

I. HEPATITIS C – An Introduction

Hepatitis C is an infectious liver disease caused by the hepatitis C virus (HCV) that in most subjects chronically infects the human liver. HCV infection is one of the major etiologies, besides hepatitis B virus (HBV) infection, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease, resulting in chronic hepatitis and progressive liver disease and thereby leading to development of lethal complications, i.e. cirrhosis and hepatocellular carcinoma (HCC), the second leading cause of cancer mortality worldwide (El-Serag and Davila 2011; Ryerson, Eheman et al. 2016).

HCV itself is mainly transmitted through direct blood-to-blood contact occurring through medical treatment with unsterilized needles, blood transfusions and more recently through needle-sharing drug abuse, also through sexual contact and mother-to-child transmission. Upon its discovery in 1989 (Choo, Kuo et al. 1989), enormous progress has been made in the field to develop diagnostic and therapeutic strategies for HCV treatment. These efforts resulted in the development of highly efficient direct-acting antivirals (DAAs) targeting specific HCV proteins and enabling cure in more than 95% of infected individuals by achieving sustained virologic response (SVR) since 2013. Indeed, the combination of different DAAs resulted in cure rates over 96% regardless of the HCV genotype of patients and with a relative short duration of treatment (Bourliere, Pietri et al. 2018). Nevertheless, major clinical and scientific challenges still remain: DAA therapy is cost-intensive and still only available to a fraction of HCV-infected patients (Chung and Baumert 2014), some infected individual developed resistance to DAAs, and an urgently needed HCV vaccine to prevent the global spread of infection is still lacking due to the high diversity of the virus, limited models for testing vaccines and a partial knowledge of protective immune response (Bailey, Barnes et al. 2019). Of note, HCC risk remains high for decades even after SVR (Morgan, Baack et al. 2013; Kanwal, Kramer et al. 2019). An estimated 71 million individuals are persistently infected with HCV with big geographical differences worldwide (WHO Global hepatitis report, 2017). The prevalence of chronic HCV infection is high, including developed countries as shown in Figure 1.

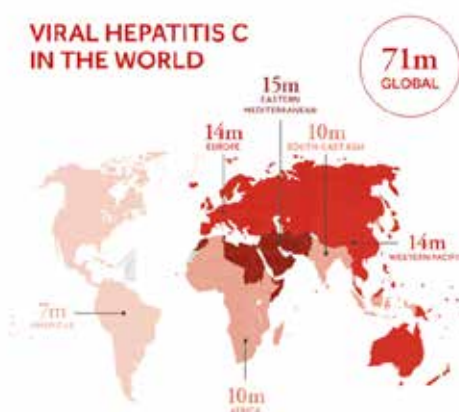


Figure 1. Estimated global HCV prevalence in 2015.

In general, there are variations in prevalence across and within countries. One could observe a higher affection of the European and Eastern Mediterranean regions by HCV infection (WHO Global Hepatitis Report, 2017).

1.1 Pathogenesis of HCV infection

Hepatitis C is caused by HCV infection of hepatocytes resulting in acute (20-30% of infected patients) or more often in chronic HCV infection (70-80% of infected patients) (reviewed in Zeisel, Cosset et al. 2008). The difference between both outcomes of infection is in principle pretty simple: Acute infections consequently lead to clearance of virus through an effective immune response mediated by HCV-specific neutralizing antibodies and T-cell responses, contrarily in chronic infections the immune system is not able to clear the virus resulting in high levels of viral replication and in dysfunctionality of the immune system (see Figure 2) (reviewed in Zeisel, Cosset et al. 2008; Park and Rehermann 2014).

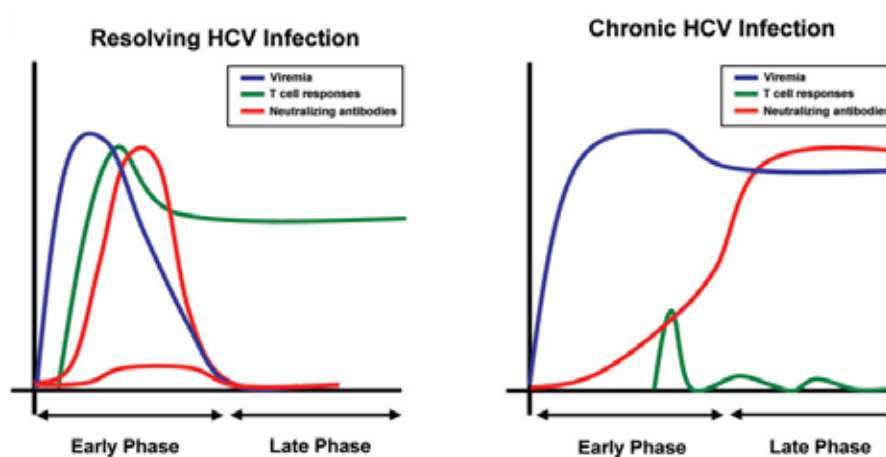


Figure 2. Acute (resolving) HCV infection versus chronic HCV infection.

While in resolving HCV infection the HCV RNA levels decrease due to effective immune responses through rapid induction of neutralizing antibodies and HCV-specific T-cell responses (left), while the contrary result can be observed during chronic HCV infection (right). (Zeisel, Cosset et al. 2008).

Obviously, chronic HCV predisposes to progressive fibrosis, cirrhosis and HCC either directly through changes of the cellular metabolism or indirectly as a result of persistent chronic inflammation.

Approximately 1-4% of patients with HCV-related cirrhosis develop HCC (NIH 2002) which is, per basic definition, a randomly occurring genomic and epigenomic change leading to an alteration of cellular gene expression and an abnormal proteome. Epigenetic and gene expression alterations observed in hepatitis C patients can be associated with high risk to HCC, even after the virus could be eradicated by treatment with DAAs (Hamdane, Juhling et al. 2019; Perez, Kaspi et al. 2019). Additionally to epigenetic changes, four pathways and biological processes can be involved in the progression of hepatocarcinogenesis: (i) oxidative stress pathways, (ii) p53 cell cycle pathways, (iii) PI3K/Akt/mTOR and MAPK pathways as well as (iv) WNT/ β -catenin pathways (Zucman-Rossi, Villanueva et al. 2015).

1.2 HCV – Basic virology

1.2.1 Genomic organization of HCV RNA

HCV, a member of the *Flaviviridae* family, is an enveloped, single-stranded positive-sense RNA virus that is mainly restricted to hepatocytes. Its genome encompasses approximately 9.6 kb encoding a single polyprotein precursor of around 3000 amino acids (aa) and is flanked by 5'- and 3'- untranslated regions (UTRs). The internal ribosome entry site (IRES), located at the 5'UTR of the genome, mediates cap-independent translation of the polyprotein contributing to an efficient translation (Tsukiyama-Kohara, Iizuka et al. 1992; Wang, Sarnow et al. 1993). Moreover, miR-122, a crucial liver-enriched host factor, binds to two target sites close to the 5'UTR promoting HCV translation (Henke, Goergen et al. 2008) and replication (Jopling, Yi et al. 2005) by stabilizing HCV RNA (Shimakami, Yamane et al. 2012) (see Figure 3).

The synthesized polyprotein is co- and post-translationally processed by host and viral proteases into at least ten viral proteins: The three structural proteins Core, and the glycoproteins E1 and E2 building up the virion as well as the seven nonstructural (NS) protein mainly involved in replication and assembly through distinct enzymatic activities, namely p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (see Figure 3).

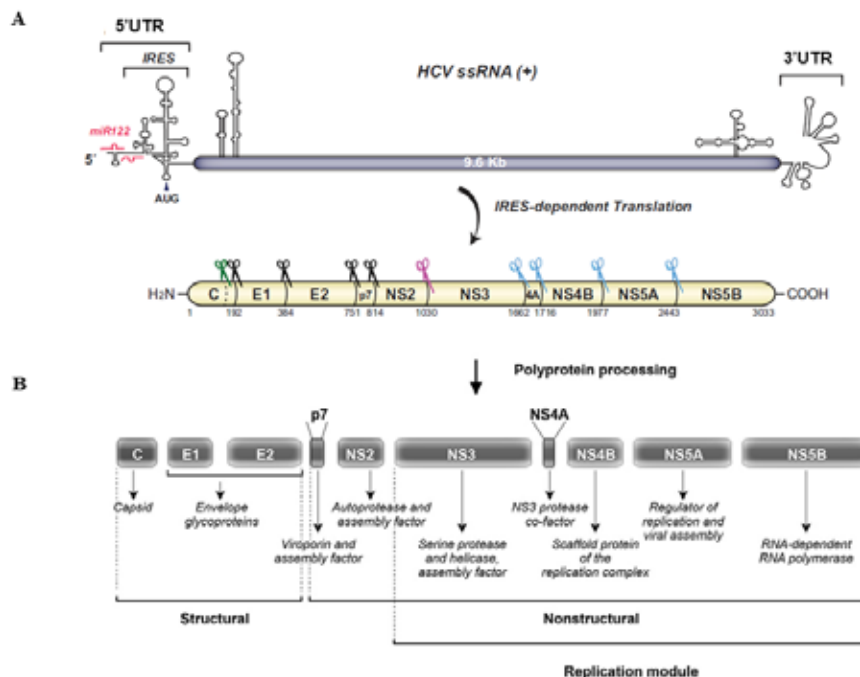


Figure 3. Overview of (A) HCV genome organization and IRES-dependent translation into polyprotein and (B) viral proteins and their function.

Scissors indicate proteolytic cleavage sites of the polyprotein. UTR: untranslated region; IRES: internal ribosome entry site; C: Core; NS: nonstructural (adapted from Dubuisson and Cosset 2014; Paul, Madan et al. 2014).

1.2.2 Genetic diversity of HCV – One challenge for vaccine design

HCV can be classified into seven genotypes and several subtypes demonstrating its high degree of genetic heterogeneity and thereby the challenges for research regarding efficient vaccine design: (i) HCV is able to rapidly mutate and (ii) to closely interact with the host lipid metabolism, both leading to escape from protective immune responses.

The genotypes (gt) are numbered from 1 to 7, whereas a variable number of sub-genotypes is designated with a lower case letter forming the basis of classifying HCV into genotypes such as 1a, 1b etc. (Simmonds, Bukh et al. 2005; Smith, Bukh et al. 2014). There is a designated distribution of all HCV strains worldwide: While genotype 1, the (from global perspective) most common genotype (almost half of all infections), dominates in Europe and the Americas, genotype 3, the second most common genotype (around 20-30% of infections), is mostly found in Asia and Northern Europe. Genotype 2 and 4 are less widespread but often found in North Africa and Middle East (around 10% of infections) (Gower, Estes et al. 2014).

1.2.3 HCV virion structure: The lipoviral particle

HCV is an enveloped virus that contains core proteins forming a nucleocapsid around the viral RNA genome which is surrounded by an endoplasmic reticulum (ER)-derived envelope with incorporated viral glycoproteins E1 and E2 which are involved in binding and entry into host cells (reviewed in Lindenbach 2013).

One outstanding characteristic of HCV particles is their tight link with the host cell lipid metabolism; even more their close association with host lipoproteins (Felmlee, Hafirassou et al. 2013; Lavie and Dubuisson 2017). To understand why one speaks of an important hallmark of the virus, it is absolutely essential to understand the process underlying the lipid homeostasis taking place in the liver: Lipids (e.g. triacylglycerides) are synthesized within the liver and their transport through aqueous medium (blood) is enabled through binding with proteins. A process in which triacylglycerides are pooled together with cholesterol and a variable number of proteins into hydrophilic lipoprotein complexes which can be subdivided upon their density into five groups: Chylomicrons (mostly generated in the intestine), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). The association of lipids with these so-called apolipoproteins (Apo) is not only important for the transport of lipids through the organism to their organs of need but also for the uptake process into cells through specific membrane receptors.

In the process of VLDL assembly, ApoB and microsomal triglyceride transfer protein (MTP) are required: After synthesis and translocation into the rough ER lumen (rER), ApoB is charged with phospholipids and cholesterol via MTP leading to formation of a neutral lipid core that is transformed into a sphere-shaped particle. This so called VLDL2 contains exchangeable ApoE and ApoC and can be released after movement to a distal compartment in the secretory pathway. Alternatively, luminal lipid droplets (luLDs) are formed as second precursors at the smooth ER (sER) following triglyceride enrichment via MTP and close association with ApoE but not with ApoB. Fusion of VLDL2 with this second precursors is leading to a triglyceride-rich lipoprotein (TLR), namely VLDL1 (reviewed in Olofsson, Bostrom et al. 2009; Shelness and Sellers 2001). Due to the differences in density, lipoproteins can be separated via ultracentrifugation.

To come back to HCV, the virus itself hijacks the host cell lipid metabolism, more particularly, parts of the VLDL and LDL secretion pathway for production of infectious virions. In fact, infectious viral particles could be found in patient-derived serum associated with VLDLs or LDLs forming a so-called lipoviral particle (LVP) (Thomssen, Bonk et al. 1992; Andre, Komurian-Pradel et al. 2002; Nielsen, Bassendine et al. 2006). As a consequence, LVPs show distinct biophysical properties than VLDLs or LDLs: (i) obviously, LVPs are rich in cholesterol and triacylglycerides displaying (very) low density, and (ii) are containing apolipoproteins (e.g. ApoB, ApoA, ApoE and ApoC) and more interestingly, (iii) patient-derived HCV particles differ in density between 1.25g/ml to below 1.06g/ml with infectivity inversely correlated to density meaning that low-density viruses are more infectious. In fact, infectious LVPs have a density between 1.03 to 1.10g/ml and thus can be separated as well as lipoproteins via density gradient ultracentrifugation (Gastaminza, Cheng et al. 2008; Piver, Boyer et al. 2017; Catanese, Uryu et al. 2013; Meunier, Russell et al. 2008) (see Figure 4). Findings that could be confirmed using *in vivo* (Nielsen, Bassendine et al. 2006; Thomssen, Bonk et al. 1992; Andre, Komurian-Pradel et al. 2002) as well as *in vitro* (Gastaminza, Kapadia et al. 2006; Lindenbach, Meuleman et al. 2006; Merz, Long et al. 2011) model systems. Interestingly, recent evidence suggests that HCV LVPs contain several exchangeable ApoE and one non-exchangeable ApoB molecule (Piver, Boyer et al. 2017).

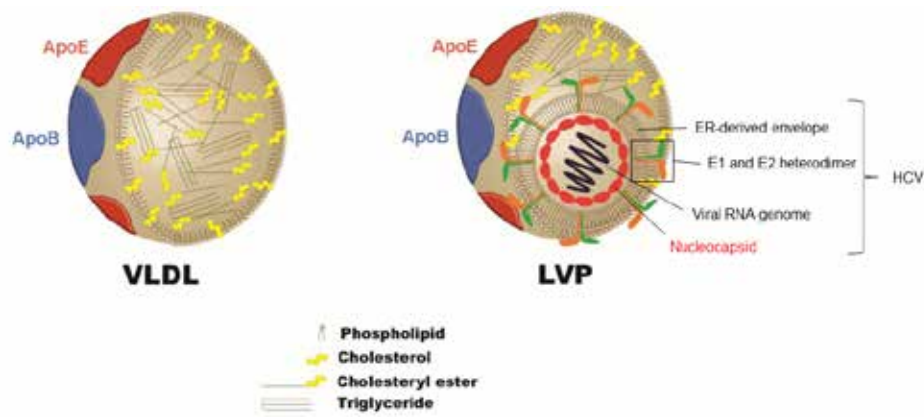


Figure 4. Infectious LVP versus VLDL.

Infectious HCV particles (LVP, right) are composed of ApoE and ApoB, which are also part of very-low-density lipoproteins (VLDL, left), and viral components. Consequently LVPs share common features with VLDLs (Felmlee, Hafirassou et al. 2013).

Consequently, through the association with VLDLs or LDLs and different kinds and amounts of apolipoproteins, HCV particles are heterogeneous and thus vary in shape and size ranging from 40 to 80 nm in diameter.

In fact, the association with ApoB and ApoE does not only lead to the masking of viral epitopes preventing the virus to be neutralized by HCV-specific antibodies (Catanese, Uryu et al. 2013; Merz, Long et al. 2011; Fauvelle, Felmlee et al. 2016) but even more this association helps to facilitate HCV entry into hepatocytes (reviewed in Zeisel, Felmlee et al. 2013).

1.2.4 The HCV life cycle

a. Virion attachment and entry

The initial step of the HCV life cycle is its attachment and entry into hepatocytes, a complex, multistep process involving both viral envelope glycoproteins E1/E2 and lipoprotein components which makes it difficult to entirely clear the exact sum of events and details that occur during this first step of the life cycle. The process itself can be subdivided into three key steps: (i) viral attachment to the hepatocyte, (ii) receptor-mediated endocytosis, and (iii) endosomal fusion.

Since LVPs are transported via bloodstream, initial attachment occurs at the basolateral membrane of the hepatocytes through binding of virion-associated ApoE to cell surface heparan sulfate proteoglycan (HSPGs) syndecan-1 or syndecan-4 and low-density lipoproteins receptor (LDLR) (Jiang, Cun et al. 2012; Shi, Jiang et al. 2013; Lefevre, Felmlee et al. 2014; Xu, Martinez et al. 2015; Grigorov, Reungoat et al. 2017).

Four main host-derived entry factors have been described, namely tetraspanin CD81 (Pileri, Uematsu et al. 1998), scavenger receptor BI (SR-BI) (Scarselli, Ansuini et al. 2002), and the tight junction proteins Claudin 1 (CLDN1) (Evans, von Hahn et al. 2007) and Occludin (OCLN) (Ploss, Evans et al. 2009), as well as numerous cofactors, especially two receptor tyrosine kinases, epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2) (Lupberger, Zeisel et al. 2011). Interactions of virion-associated ApoB-100 with SR-BI is proposed to induce lipoprotein-HCV dissociation (Scarselli, Ansuini et al. 2002; Dreux, Dao Thi et al. 2009; Maillard, Huby et al. 2006) leading to direct interaction of HCV glycoprotein E2 with SR-BI and CD81 (Dao Thi, Granier et al. 2012; Scarselli, Ansuini et al. 2002; Pileri, Uematsu et al. 1998; Bartosch, Vitelli et al. 2003). At this stage of HCV entry, additional entry factors are required: CLDN1 and OCLN (Evans, von Hahn et al. 2007; Ploss, Evans et al. 2009; Liu, Yang, Shen et al. 2009). Two models have been proposed. Recently, imaging-based studies in a three-dimensional polarized hepatoma system reported that an initial co-localization of HCV with SR-BI, CD81 and EGFR at the basolateral membrane occurs, leading to trafficking and accumulation of HCV virions at the tight junctions, where the interaction with CLDN1 and OCLN takes place (Baktash, Madhav et al. 2018). Furthermore, HCV may also interact with CLDN1 on the basolateral membrane of hepatocytes. Indeed, although CLDN1 and OCLN are classified as tight junction proteins, a minority of these proteins can be found on the basolateral membrane (reviewed in Zeisel, Dhawan et al. 2018). Using a CLDN1-targeting monoclonal antibody (mAb) and confocal microscopy, Maily et al could observe a minority pool of CLDN1 found on basolateral membrane of hepatocytes in chimeric mouse liver as well as in normal liver tissue (Maily, Xiao et al. 2015). Interestingly, fluorescence resonance energy transfer-based studies could show that using CLDN1-specific mAb leads to the perturbation of CD81-CLDN1 co-receptor formation at hepatocyte basolateral membrane and inhibition of HCV entry (Maily, Xiao et al. 2015; Fofana, Krieger et al. 2010).

Host cell kinases have been shown to contribute to the regulation of viral entry by promoting co-receptor association between CD81 and CLDN1 which is essential for HCV entry (Harris, Farquhar et al. 2008; Harris, Davis et al. 2010; Farquhar, Harris et al. 2008; Lupberger, Zeisel et al. 2011). Indeed, inhibition of EGFR and EphA2 via the protein kinase inhibitors erlotinib and dasatinib, respectively, led to disruption of CD81-CLDN1 co-receptor formation resulting in inhibition of HCV entry (Lupberger, Zeisel et al. 2011). These results propose the direct contribution of EGFR and EphA2 in CD81-CLDN1 co-receptor complex (Lupberger, Zeisel et al. 2011) and moreover, the identification of HRas, a GTPase acting downstream of EGFR signaling, and its association with CD81 and CLDN1 supports the model that kinase signaling pathways play a role in this formation process (Zona, Lupberger et al. 2013). In fact, CD81-CLDN1 complex formation could also be disrupted using a protein kinase A inhibitor leading to an intracellular localization of CLDN1 and

consequently to an impaired viral entry (Farquhar, Harris et al. 2008). It could also be shown that binding to CD81 triggers the autophosphorylation of EGFR (Diao, Pantua et al. 2012), resulting in basolateral diffusion of CD81; which in turn leads to an association with CLDN1 and the formation of the CD81-CLDN1 co-receptor complex (Harris, Farquhar et al. 2008; Harris, Davis et al. 2010). In contrast to CD81, SR-BI and CLDN1, the role of OCLN in the viral entry process has been less well studied. Nonetheless, one group was able to create mutants of OCLN proteins blocking HCV cell entry via specific antibodies. It could be demonstrated that OCLN is required in late steps of HCV entry and may be directly interacting with HCV virions *in vitro* (Sourisseau, Michta et al. 2013). Indeed, Shimizu et al. were able to develop functional mAbs directed against extracellular domains of OCLN confirming that OCLN is required in late steps of HCV entry, and inhibition of OCLN via mAbs resulted in inhibition of HCV infection *in vitro* and *in vivo*. Moreover, by using these mAbs HCV cell-free and cell-to-cell transmission was efficiently blocked (Shimizu, Shirasago et al. 2018). CD81-CLDN1 bound HCV virions are internalized via clathrin- and dynamin-dependent endocytosis (Blanchard, Belouzard et al. 2006; Farquhar, Hu et al. 2012), a process in which EGFR-signaling appears to be required (Baktash, Madhav et al. 2018).

These processes describe the complex mechanism of cell-free HCV entry, while an alternative route of HCV entry is cell-cell transmission (Timpe, Stamataki et al. 2008; Brimacombe, Grove et al. 2011). In contrast to cell-free HCV entry, HCV cell-cell transmission is insensitive to most neutralizing antibodies and thus represents the main mode of viral spread. There is an overlap of host factors required for both entry routes; however, while cell-free HCV entry is strictly dependent on CD81 interactions, cell-cell transmission can also occur in a CD81-independent mode of action (Witteveldt, Evans et al. 2009; Jones, Catanese et al. 2010). Consequently, HCV-infected hepatocytes are found in discrete clusters inside the liver in an abundance of one to fifty-four percentage of all hepatocytes (Wieland, Makowska et al. 2014).

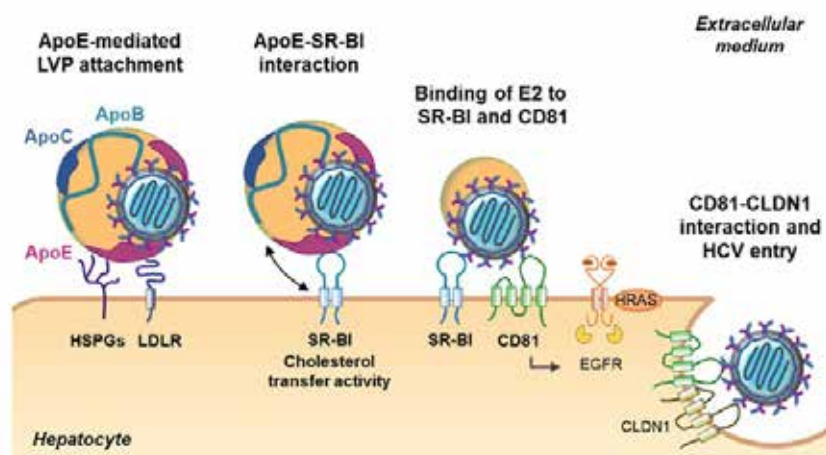


Figure 5. HCV attachment and entry into hepatocytes (Wrensch, Crouchet et al. 2018).

Membrane fusion is mediated by viral glycoproteins E1 and E2, after acidification of HCV-bearing endosomes leading to uncoating and cytoplasmic release of the genome (Douam, Dao Thi et al. 2014; Lavillette, Bartosch et al. 2006; Sharma, Mateu et al. 2011).

b. Genome translation and co-translational processing

Upon its release into the cytoplasm, the positive-strand HCV RNA genome directly serves as a template for viral polyprotein synthesis at the rough ER. Basically, a polyprotein precursor of 3000 aa in length is synthesized and co- and post-translationally cleaved by cellular (e.g. signal peptidases) and viral proteases (NS2, NS3-4A heterodimer) into 10 mature viral proteins.

In contrast to eukaryotic messenger RNA (mRNA), HCV RNA genome lacks a 5'-terminal cap and a 3'-terminal poly(A) tail. To overcome this genetic differences, the 5'UTR shows some special structural finesse: (i) the presence of a functional IRES within the 5'UTR of the viral genome allows to anchor in the ribosome and ensure cap-independent translation initiation (Lukavsky 2009), and (ii) the direct targeting of liver-enriched miR-122, on two target sites within the 5'UTR as well as three additional target sites in the coding region and the 3'UTR, stabilizes HCV RNA and contributes to HCV translation and replication (Niepmann, Shalamova et al. 2018; Jopling, Yi et al. 2005); even more, this interaction prevents degradation of the viral genome by host degradation machinery through exo- and endonucleases (reviewed in Li, Yamane et al. 2015). miRNAs are a class of small non-coding RNA molecules that normally target specific mRNA by base-pairing with a complementary site typically located at the 3'UTR, thus post-transcriptionally regulating gene expression (Saliminejad, Khorram Khorshid et al. 2019). In fact, miR-122 acts completely contrary to normal destabilizing actions of miRNAs on host mRNAs by the binding and stabilization of the 5'UTR of HCV RNA genome and thus promoting viral replication and persistence (reviewed in Li, Yamane et al. 2015).

After initiation of translation, protein synthesis is blocked by a signal sequence between core and E1 targeting the ribosome to the translocon complex of the ER where translation proceeds. The nascent polypeptide is cleaved by the signal peptidases of the ER and the 191 aa core precursor is released into the cytosolic side of the ER (Santolini, Migliaccio et al. 1994) and further processed into the mature core protein of 21 kDa size by an ER-residing cellular protease (McLauchlan, Lemberg et al. 2002). E1 and E2 are the next viral proteins that are released from the polyprotein precursor after cleavage by host signal peptidases, both containing a large N-terminal ectodomain and a C-terminal hydrophobic anchor (Dubuisson 2000). E1 and E2 assemble to form a non-covalent heterodimer (Dubuisson 2000); additionally, the C-termini of both glycoproteins are co-translationally integrated

and anchored into the ER membrane resulting in protruding of their ectodomains into the ER lumen (Cocquerel, Op de Beeck et al. 2002). NS2 is able to release itself from the polyprotein by its autocatalytic activity (Grakoui, McCourt et al. 1993), while the remaining nonstructural proteins are cleaved by NS3-4A protease (Tomei, Failla et al. 1993). All nonstructural viral proteins are anchored within the ER and oriented towards the cytosolic side.

Of note, one could see miR-122 as well as the presence and expression rates of the HCV entry factors (see p.7) and the close association with host-derived lipoproteins (see p.5) as possible reasons explaining the strict hepatotropism of HCV (Dubuisson and Cosset 2014).

c. Genome replication

Importantly, the HCV genome does not integrate into the host genome and therefore continuous replication of the viral genome is required for the maintenance of chronic infection. After processing of the viral proteins, the nonstructural proteins NS3 to NS5B induce distinct membrane alterations that contain the sites of viral RNA replication.

Through this enormous rearrangements of intracellular ER membranes, the HCV replication complex, also known as membranous web, is formed by activity of viral NS4B (Gouttenoire, Penin et al. 2010) and of the cellular lipid kinase phosphatidylinositol-4-kinase (PI4K α), whose lipid kinase activity is initiated by interaction with NS5A and NS5B (Paul, Hoppe et al. 2013; Ferraris, Beaumont et al. 2013; Reiss, Rebhan et al. 2011). Additionally, the activated replication machinery also requires NS4A acting as a co-factor forming a heterodimer with NS3 and triggering NS3 protease function (Bartenschlager, Lohmann et al. 1995), and host factor cyclophilin A (CyPA) (Liu, Yang, Robotham et al. 2009; Kaul, Stauffer et al. 2009). Of note, the viral RNA-dependent RNA polymerase, NS5B, plays the key role in HCV RNA synthesis (Lohmann, Korner et al. 1997; Behrens, Tomei et al. 1996). The membranous web is a typical morphological feature of positive-strand RNA viruses (Miller and Krijnse-Locker 2008), and can be visualized via electron microscopy of HCV-infected hepatocytes, often in close proximity with lipid droplets (LDs) indicating the tight link of HCV with host cell lipid metabolism (see Figure 6); indeed HCV core (McLauchlan, Lemberg et al. 2002) and NS5A (Shi, Polyak et al. 2002) could be found in association with LDs implicating a possibly key role in coordination of viral replication and virion morphogenesis (Bartenschlager, Penin et al. 2011) (see p.13).

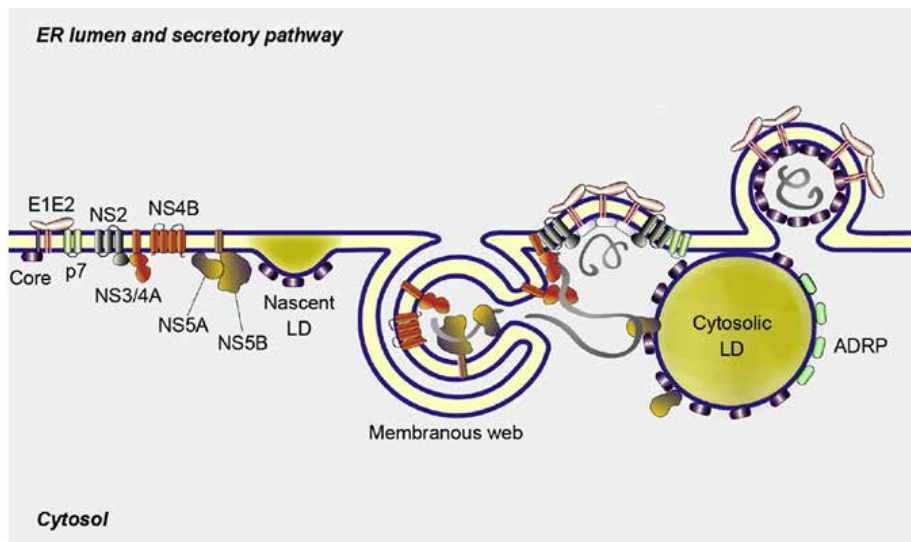


Figure 6. HCV replication and formation of the membranous web (adapted from Dubuisson and Cosset 2014).

In the primer-independent initiation step of RNA replication, a negative-strand of the genome is generated serving as template for progeny positive-strand viral genomes. After formation of a dinucleotide that acts as a primer, elongation of the nascent negative-strand RNA chain occurs mediated by NS5B proceeding in a 3'-to-5' direction resulting in a positive-strand RNA molecule without the help of other factors (Lohmann 2013). About termination only little is known, but one can imagine that the polymerase dissociates from the template after reaching its end. Of note, the polymerase NS5B does not work properly, but rather error-prone resulting in high genetic variability of HCV isolates. The newly generated positive-strand RNA copies either serve as template for continuing viral protein synthesis or move to LDs resulting in assembly of progeny virions or they remain within the membranous web undergoing negative-strand RNA synthesis for replication (Lohmann 2013). HCV is able to create an environment conducive to its replication and assembly: the formation of the membranous web is one example. Moreover, the ratio of neutral to membrane lipids is reduced upon HCV infection and membrane lipids as cholesterol and phospholipids were gathered in microsomal fractions of HCV-infected cells (Hofmann, Krajewski et al. 2018). Additionally, HCV seems to recruit various cytoplasmic nuclear pore complexes (Nups) to site of replication where they could be found in increased numbers and accumulated at the membranous web and even more co-localized with core or NS5A (Levin, Neufeldt et al. 2014; Neufeldt, Joyce et al. 2013). It is proposed that cytoplasmic Nups form channels across the double membrane structures of the membranous web to serve as gatekeepers by facilitating movement of several HCV proteins and host proteins without being recognized by the pattern recognition receptors (PRRs) of the innate immune system (see Figure 7) (Levin, Neufeldt et al. 2014; Neufeldt, Joyce et al. 2013). Upon Dengue and hepatitis A virus (HAV) infection, a similar redistribution of cytoplasmic Nups could be observed

implicating a possible conserved role of cytoplasmic Nups upon positive-strand RNA virus infections (Neufeldt, Joyce et al. 2013).

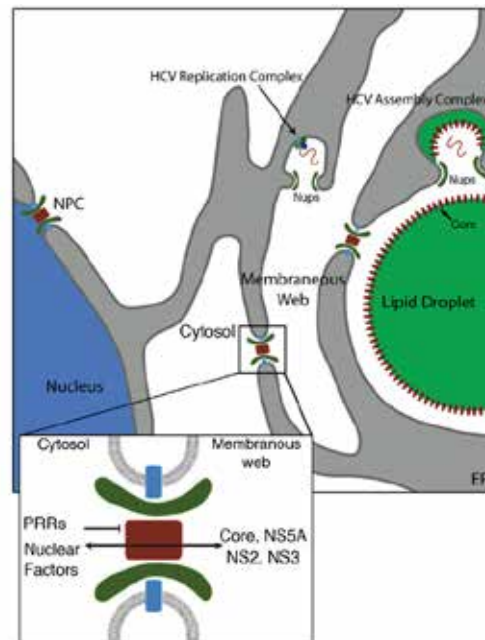


Figure 7. Potential function of the cytosolic nuclear pore complexes in HCV replication and assembly (Neufeldt, Joyce et al. 2013).

d. Viral assembly and egress

After replication of the viral genome, RNA progeny can be used for encapsulation into new virions which can be released using a noncytolytic pathway related to the VLDL secretory pathway (Chang, Jiang et al. 2007; Jiang and Luo 2009; Gastaminza, Cheng et al. 2008; Huang, Sun et al. 2007). Several viral and host proteins are involved in the viral assembly process that can be divided into an early and late phase of assembly, whereupon detailed information on different steps is still lacking. During the early phase, the nucleocapsid is formed by the involvement of different recruitment processes with the aim to localize in close proximity to LDs and to the assembly site in the ER lumen: (i) Shuttle between HCV core protein and LDs, (ii) movement of the replication complex through action of NS5A, (iii) recruitment of glycoproteins E1 and E2 by interaction with NS2 (reviewed in Popescu, Riva et al. 2014).

HCV core protein attaches to LDs through its hydrophobic domain, replacing adipose differentiation-related protein (ADRP, Figure 6) leading to an accumulation of LDs in perinuclear regions (Boulant, Douglas et al. 2008; Boulant, Montserret et al. 2006). In terms of assembly efficiency, core-LD association is a crucial step resulting in downstream recruitment of viral proteins (Miyanari, Atsuzawa et al. 2007). However, an inverse correlation of core-LD motility and assembly efficacy of

HCV was determined implicating that core is see-sawing from LD-ER interface to the ER or *vice-versa* (Shavinskaya, Boulant et al. 2007). Diacylglycerol acyltransferase 1 (DGAT1) is the key host factor involved in this step of assembly by facilitating recruitment of core onto the LD by direct core-DGAT1 interaction (Herker, Harris et al. 2010).

Since HCV RNA replication takes place within the membranous web, two possible models are proposed where assembly starts at ER membrane or at the surface of cytosolic LDs (cLDs): (1) Transfer of HCV core protein onto the surface of cLDs and re-recruitment to ER membrane at assembly sites where interaction of core with NS5A occurs concluding that cLDs might serve as trafficking vehicles to transport core from sites of translation and replication to sites of viral assembly; or (2) Initiation of nucleocapsid formation occurs on the surface of cLDs, while distributions of HCV RNA to core protein is mediated by NS5A that is colocalized onto surface of cLDs. Currently, discrimination between both hypotheses is not easy by considering the low assembly efficiency of HCV per cell (reviewed in Bartenschlager, Penin et al. 2011).

NS5A as well as DGAT1 seem to play a central role in the early stage of viral assembly. Indeed, an interaction between the hyperphosphorylated form of NS5A and core could be observed leading to the relocation of NS5A to LDs (Masaki, Matsunaga et al. 2014), while the hypophosphorylated form of NS5A correlates with genomic replication (Evans, Rice et al. 2004). Given the direct interaction of core-DGAT1, also NS5A directly interacts with DGAT1 resulting in its recruitment to LDs (Camus, Herker et al. 2013). Additionally, an interaction between NS5A and ApoE is also suggested to contribute to the recruitment of this apolipoprotein to assembly sites (Benga, Krieger et al. 2010). Besides, NS5A was also reported to interact with Rab18, a small G protein, to facilitate its recruitment to LD and HCV assembly site (Salloum, Wang et al. 2013). The transition from replication to assembly is represented by the recruitment of NS5A together with the replication complex to LDs (reviewed in Lindenbach and Rice 2013).

The last step of the early phase, and in fact the first step of late phase, of viral assembly is the recruitment of envelope proteins at assembly sites. Several groups reported that the viral envelope glycoproteins E1 and E2 are bound in complexes composed of NS2, p7 and NS3 (Jirasko, Montserret et al. 2010; Popescu, Callens et al. 2011; Stapleford and Lindenbach 2011; Ma, Anantpadma et al. 2011) resulting in an accumulation of these complexes in close proximity of NS5A, core and LDs (Jirasko, Montserret et al. 2010; Popescu, Callens et al. 2011). However, the concrete nature and composition of this complex is not yet fully understood, however, it could be determined that NS2 and HCV E2 are associated with detergent-resistant membranes (DRMs) which are required for efficient HCV assembly (Shanmugam, Saravanabalaji et al. 2015); moreover, the entire replication complex as well as the HCV structural proteins could be shown to be associated with those DRMs

(Aizaki, Morikawa et al. 2008; Aizaki, Lee et al. 2004; Paul, Hoppe et al. 2013; Shi, Polyak et al. 2002). Recently, Boyer et al had a deeper look on the nature of this NS2 complex and found a direct protein-protein interaction of NS2 and E1E2 using immunoprecipitation assays revealing an association of NS2 with NS3 via DRMs. Contrarily, NS5A and E1E2 do not associate but rather interact separately with NS2-E1-E2-NS3 complex via unstable DMRs (Boyer, Dreneau et al. 2019). Interestingly, core was found to interact with NS2 and E1E2 through unstable DMRs suggesting a crucial role of DMRs transporting the NS2-E1-E2-NS3-NS5A-core complex for HCV assembly initiation (Boyer, Dreneau et al. 2019).

The late phase of viral assembly is the maturation and release of HCV particles in tight association with the host VLDL pathway within the ER lumen (Chang, Jiang et al. 2007; Jiang and Luo 2009; Gastaminza, Cheng et al. 2008; Huang, Sun et al. 2007). Indeed, it could be shown that HCV core accumulates in lipid fractions which contain high amounts of cholesterol and sphingolipids (Matto, Rice et al. 2004); these two lipids are observed to be enriched in viral particles and in turn involved in their infectivity (Aizaki, Morikawa et al. 2008). The nucleocapsid could be integrated into the core of luminal LDs (luLDs); however, how E1 and E2 are integrated into virions remains unclear. It is proposed that there is an interaction between NS3, E1 and E2 by either NS2 alone or together with p7 (Yi, Ma et al. 2009; Phan, Beran et al. 2009). Indeed, two studies observed that NS2 brings together E1, E2, p7, NS3 and NS5A in close proximity of LD by protein-protein interactions between all involved components (Jirasko, Montserret et al. 2010; Ma, Anantpadma et al. 2011). Since HCV E2 is the well-studied HCV glycoprotein that plays the major role for neutralization of HCV-specific antibodies and the binding capacity to CD81, the involvement of HCV E1 in HCV replication cycle still remains to be elucidated. By studying the role of E1 for viral morphogenesis, Haddad et al used mutants of E1 observing first a shift in the receptor usage from CLDN1 to CLDN6 for two mutants, and second virus carrying one mutant (D263A) was assembled and released but devoid of viral RNA revealing a crucial role for E1 in incorporating HCV RNA into the nucleocapsid (Haddad, Rouille et al. 2017). The step in which the apolipoproteins incorporate into mature infectious particles is also not fully clear. It is assumed that ApoB-positive precursors, formed in the rough ER (rER), fuse together with ApoB-negative precursor which incorporate the viral nucleocapsid resulting in LVPs associated with nonexchangeable ApoB (see p.5). ApoE and ApoC, both exchangeable apolipoproteins, could be associated to the LVPs within the ER lumen. However, there are differences between HCV particles depending in which cell type they are produced (detailed comparison is found on p.20).

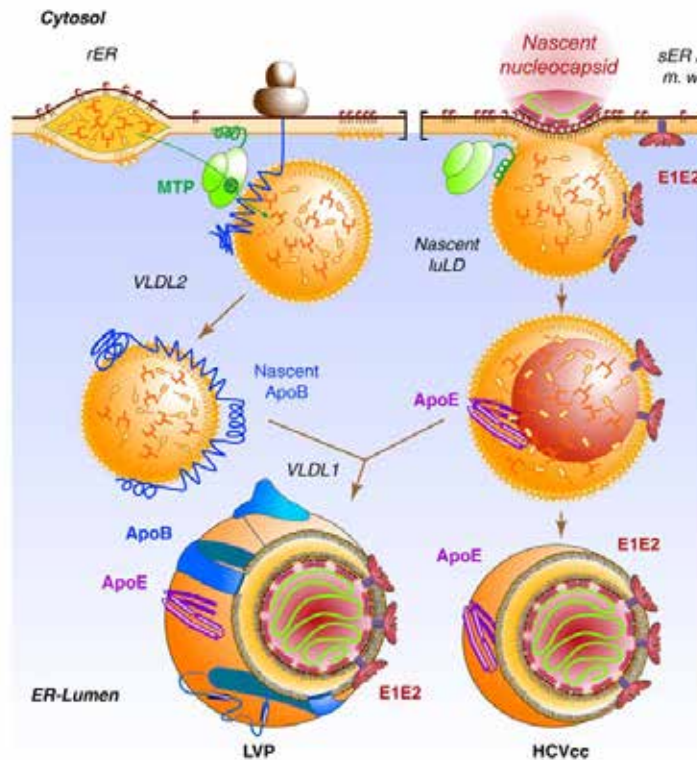


Figure 8. Model of the formation of LVPs and comparison to cell culture-derived HCV (HCVcc).

Precursors are formed: (1) VLDL2 resulted from the translocation of nascent ApoB into the ER lumen and the association with phospholipids, triglycerides and ApoE/C which are not shown for clarity (left), (2) luminal LD (luLD) is generated at the smooth ER (sER) or the membranous web (m.w.) and enriched with triglycerides via MTP (right). E1 and E2 associate with luLD after release from the ER membrane and the nucleocapsid is proposed to insert within the hydrophobic core of the luLD. In primary human hepatocytes the nucleocapsid-loaded luLD fuses with the VLDL2 resulting in a LVP, while in the cell culture model VLDL1 formation is lacking, cell culture-derived (HCVcc) is released without ApoB association (more details of in vitro models see on p.20). For clarity reasons, VLDL1 is generated as a fusion of VLDL with the triglyceride-rich luLD (Bartenschlager, Penin et al. 2011).

LVPs are transported along the VLDL secretory pathway (reviewed in Bartenschlager, Penin et al. 2011) to the Golgi, where the HCV glycoproteins E1 and E2 are post-translationally modified (reviewed in Vieyres, Dubuisson et al. 2014). The exact mechanism of HCV budding at the plasma membrane is still lacking. Immunoprecipitation and electron microscopy (EM) analysis revealed that HCV LVPs assemble in the ER and are transported to Golgi through vesicular transport mediated by COPII vesicles to enter the Golgi secretory pathway (Syed, Khan et al. 2017). In line with these findings, Takacs et al could observe that the secretion of ApoE and ApoB is differentially controlled by Rab1b which is a major regulator of transport from the ER to Golgi, and thus regulates secretion of HCV as well (Takacs, Andreo et al. 2017). Golgi protein 73 (GP73) has been demonstrated to be involved in HCV secretion: normally as a Golgi membrane resident protein and upregulated upon HCV infection, GP73 is colocalized with the HCV replication complex in Huh7.5.1 cells and is able to directly mediate an interaction of NS5A and ApoE and thus promoting the secretion of HCV (Zhang, Wang et al. 2016). These studies all supported the idea that HCV secretion and budding occurs through the Golgi secretion pathway. However, on the other hand could be demonstrated that

HCV is hijacking proteins of the endosomal sorting complex required for transport (ESCRT), e.g. hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), for budding at the plasma membrane. Indeed, HRS is able to directly interact with NS2 and NS5A in HCV-infected cells supporting efficient viral assembly as well as viral budding (Barouch-Bentov, Neveu et al. 2016). In line with this, another group found viral particles and structural proteins in endosomal compartments but not in compartments of the Golgi assuming that release of HCV occurs through noncanonical secretory route which is different from the canonical Golgi-mediated secretion (Bayer, Banning et al. 2016).

In sum, the above described processes followed the hypothesis that virions exist as hybrid lipoviral particles protecting from neutralization with HCV-specific antibodies (Andre, Komurian-Pradel et al. 2002). However, in a proposed two-particle model, the virus and serum lipoproteins stably interact as separate particles via protein-protein interaction. Indeed, when cholesterol is chemically removed from HCVcc particles their infectivity is lost, while adding back exogenous cholesterol led to restorage (Aizaki, Morikawa et al. 2008). Additionally, another group could observe a rapid shift of buoyant density of viral particles in serum of fasting HCV patients concluding that virions and serum lipoproteins associate in a transient and exchangeable way (Felmlee, Sheridan et al. 2010). Both hypotheses could be imaginable, but they still need to be confirmed.

1.3 Model systems to study HCV

1.3.1 *In vitro* model systems

After its discovery in 1989 (Choo, Kuo et al. 1989), several efforts to culture HCV *in vitro* failed through lacking robustness, inefficient replication and thus failure for detailed molecular studies of the viral life cycle (reviewed in Bartenschlager and Lohmann 2000). After ten years of research, several breakthrough developments led to improvement to study the virus *in vitro*: the replicon system in 1999 followed by retroviral pseudoparticles in 2003 and finally the cell culture system recapitulating the entire viral lifecycle in 2005 (see Figure 9).

a. HCV replicon system

The establishment of sub-genomic replicons that autonomously amplify in cultured human hepatoma cells was a first major breakthrough to study viral replication: the genotype 1b HCV genome that was derived from a chronically infected patient was trimmed to those components essential for replication (NS3 to NS5B), while the viral structural genes (core, E1, E2) as well as p7 and NS2 were deleted

(see Figure 9). Practically, this shortened HCV genome was cloned into a plasmid resulting in a replicon encoding the 5'UTR of HCV, the first 12 codons of the core protein fused with a G418 selection cassette, the IRES from encephalomyocarditis virus (EMCV) allowing translation of the non-structural proteins, as well as viral NS3, NS4A, NS4B, NS5A, NS5B, and 3'UTR. After undergoing *in vitro* transfection, the construct is transfected into human hepatoma 7 (Huh7) cells (see p.20) resulting in direct translation of viral RNA and G418 selection of those Huh7 cells displaying high levels of HCV RNA replication (Lohmann, Korner et al. 1999). Subsequently, full length HCV replicons were developed, ultimately leading to the establishment of recombinant full-length infectious HCV (see HCVcc below).

b. HCV Pseudoparticles (HCVpp)

Four years after developing the replicon system, the next important achievement was the generation of retroviral pseudoparticles displaying functional HCV glycoproteins, E1 and E2, for dissection of HCV entry process. In basic principal, pseudoparticles are retroviral capsids with incorporated viral glycoproteins in their envelopes. Consequently, these pseudoparticles allow to study the relevance and need of those glycoproteins in viral attachment and entry processes; the latter can be easily quantified using a reporter gene located inside the pseudoparticles. HCVpp could be successfully developed by integrating HCV glycoproteins in retroviral particles: A system in which 293T cells are co-transfected with expression vectors encoding HCV E1 and E2, the gag-pol proteins of either murine leukemia virus (MLV) or human immunodeficiency virus (HIV), and a retroviral genome encoding a reporter gene such as green fluorescent protein (GFP) or luciferase (Bartosch, Dubuisson et al. 2003; Hsu, Zhang et al. 2003) (see Figure 9). Using HCVpp is an elegant way to study HCV entry independently of the entire viral life cycle which in turn highlights the limitation of HCVpp to the entry process: they are produced in a non-liver cell line and thus assemble as retroviruses on plasma membranes without getting associated with lipoproteins, contrarily liver-generated HCV particles assemble in the ER in close association with lipoproteins (see p.5 and 13).

c. Cell culture-derived HCV (HCVcc)

After successful development of sub-genomic replicons and the HCVpp system, the challenge to establish an HCV permissive cell culture system remained.

The initial production of cell culture-derived HCV particles is based on the genotype 2a HCV strain, namely JFH1 (Japanese Fulminant Hepatitis 1) that was isolated from a Japanese patient with acute viral hepatitis (Kato, Furusaka et al. 2001). Based on the wild-type JFH1 genome or chimeras consisting of the JFH1 replicase genes (NS3 to NS5B) and core to NS2 regions of alternative HCV

genomes, three groups proposed a robust *in vitro* system recapitulating the entire HCV life cycle in Huh7 cells (Lindenbach, Evans et al. 2005; Wakita, Pietschmann et al. 2005; Zhong, Gastaminza et al. 2005) which since then is the laboratory standard for *in vitro* HCV studies. These produced particles, well-known as cell culture-derived HCV (HCVcc), are able to infect new target cells, thereby completing the entire HCV life cycle (see Figure 9). This achievement led to the development of several chimeras that consist of JFH1 replicase genes NS3 to NS5B and core to NS2 of alternative HCV genomes allowing to study HCV entry, neutralization and assembly of all seven known HCV genotypes. Among all these chimeras, Jc1, consisting of the JFH1 nonstructural proteins (NS3 to NS5B) and core-E1-E2-p7 and partially NS2 of J6 (HCV gt 2a), produces infectious virus titers that are around 100 to 1000-fold higher than the original JFH1 strain (Pietschmann, Kaul et al. 2006). Over the years, chimeras of all seven known genotypes of HCV based on JFH1 have been developed giving the possibility to study the entire viral life cycle and additionally, neutralization via HCV-specific antibodies (Gottwein, Jensen et al. 2011; Gottwein, Scheel et al. 2009; Scheel, Gottwein et al. 2008; Scheel, Gottwein et al. 2011; Jensen, Gottwein et al. 2008; Gottwein, Scheel et al. 2007), however, with differences concerning infectivity. In order to rapidly and efficiently detect viral replication and infection, reporter genomes of different HCV chimeric genomes containing luciferase or GFP were generated (reviewed in Vieyres and Pietschmann 2013). Furthermore, non JFH1-based HCVcc enabling the production of recombinant HCV of different genotypes have been established but JFH1-based HCVcc remain the most widely used and efficient models.

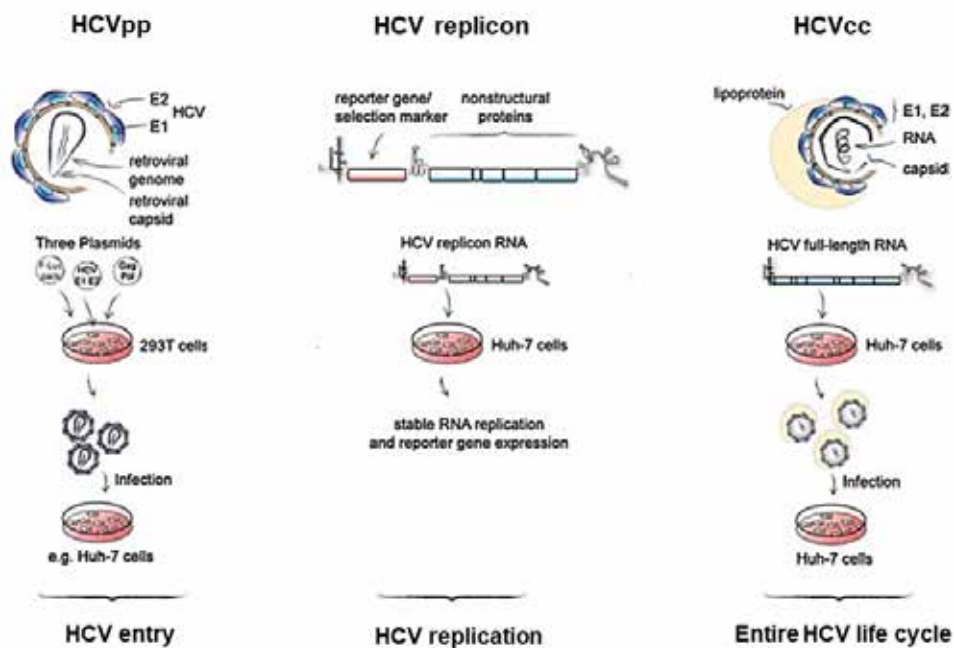


Figure 9 *In vitro* HCV cell culture models.

In vitro HCV cell culture models to investigate different steps of the viral life cycle: HCV pseudoparticle system (left) to study viral entry, the HCV replicon (middle) system to study viral replication, and full-length recombinant cell culture-derived HCV (right) system to investigate the entire viral replication cycle. (Adapted from Steinmann and Pietschmann 2013).

d. HCV-permissive host cells

Since HCV primarily replicates in human hepatocytes, cultured primary human hepatocytes (PHH) from liver resection of patients represent the most physiological *in vitro* model closest to the natural host (Ploss, Khetani et al. 2010). However, PHH display several disadvantages that make them not easy to handle in laboratories: difficult to obtain, high donor-dependent variability and limited time of culture (time-dependent dedifferentiation and approximately two weeks before apoptosis occurs); even worse, once infected with HCV PHH only show low-level replication (Fournier, Sureau et al. 1998; Molina, Castet et al. 2008; Rumin, Berthillon et al. 1999), hence making it difficult to conduct complex experiments.

Thus, the most HCV-permissive and widely used cell line are Huh7 human hepatoma cells that were originally isolated from HCC of a Japanese patient (Nakabayashi, Taketa et al. 1982). Several Huh7 subclones with increased HCV permissiveness could be obtained, by selection of replicon-containing Huh7 cells. The subclones Huh7.5 (Blight, McKeating et al. 2002), Huh7.5.1 (Zhong, Gastaminza et al. 2005), and Huh7-Lunet (Friebe, Boudet et al. 2005) cells were obtained after cure of the HCV-transfected Huh7 cells from the replicon via treatment with INF- α , INF- γ or selective inhibitors. It is not entirely clear why these Huh7 subclones yield higher levels of HCV RNA replication. However, in case of Huh7.5 cells mutations in the retinoic acid-inducible gene I (RIG-I) were shown to be involved in increased viral replication (Sumpter, Loo et al. 2005).

However, Huh7-derived cells are dedifferentiated and asynchronously dividing cancer cells, in contrast to primary hepatocytes which are differentiated and quiescent (Michalopoulos and DeFrances 1997). However, they show hepatocyte-specific gene expression and arrested cell growth by adding 1% of dimethyl sulfoxide (DMSO) to the culture medium, while permissiveness to HCV is maintained (Sainz and Chisari 2006); and thus are closer to hepatocytes.

Importantly, cultured Huh7-derived cells and *in vivo* hepatocytes show differences in their capability in producing lipoproteins, and thus HCV particles differ in their properties in dependency of the cells in which they are produced. In infected patients, the heterogenous density of circulating HCV particles and their infectivity is negatively correlated meaning low-density viruses are more infectious (Bradley, McCaustland et al. 1991; Hijikata, Shimizu et al. 1993). Those HCV particles can be immunoprecipitated via ApoB-specific antibodies confirming the association of HCV with triglyceride-rich lipoproteins (TRLs) within hepatocytes and their circulation via the bloodstream as LVPs (Andre, Perlemuter et al. 2005; Nielsen, Bassendine et al. 2004). In comparison to patient-derived LVPs, the negative correlation for density and infectivity could also been shown for HCVcc (Miyinari, Atsuzawa et al. 2007; Podevin, Carpentier et al. 2010; Haid, Pietschmann et al. 2009). Nevertheless, HCVcc were shown to be captured via ApoE (Chang, Jiang et al. 2007; Merz, Long et

al. 2011) - and not ApoB-specific antibodies (Merz, Long et al. 2011; Huang, Sun et al. 2007) highlighting the tight association of HCVcc with ApoE and ApoC and the inefficiency of Huh7-derived cells in producing authentic VLDL (reviewed in Bartenschlager, Penin et al. 2011; Vieyres and Pietschmann 2019). However, Piver et al described that at least a small fraction of HCVcc derived from Huh7.5 cells displayed ApoB and ApoE as well as E1E2 on the surface after immunogold staining following immunocapture (Piver, Boyer et al. 2017). Recently, it was confirmed that Huh7.5 cells growing in serum-free medium produced immature HCV particles, however, when incubating those cells/particles with physiological serum conditions and concentrations of lipoproteins resulted in the maturation of HCV particles to fully lipidated and notably, ApoB-containing infectious virions displaying low density very similar to patient-derived LVPs (Denolly, Granier et al. 2019). In addition to HCV maturation through the cell secretory pathway, also extracellular lipidation of particles may occur through serum itself properly after egress (Denolly, Granier et al. 2019). In contrast, HCV nucleocapsids derived from patients and cell culture appeared to be similar in size and structure and interestingly those nucleocapsids are found to be surrounded by an irregular, detergent-sensitive crescent which may be consistent with lipids (Piver, Boyer et al. 2017).

1.3.2 *In vivo* model systems

Cell culture model systems to study HCV are of high importance, however, the complex immunological host responses and liver disease progression cannot be fully answered *in vitro*. Since lacking control parameters such as time and dose of infection strongly limited clinical research in patients, attention of research was directed to animal models such as chimpanzees, tree shrews and rodents by keeping in mind that ideally, similarly to HCV-infected patients, HCV-infected animals should develop chronicity followed by liver disease progression towards cirrhosis and HCC.

Chimpanzees (*Pan troglodytes*) are with more than 98% genetic identity the closest living relatives to human and susceptible to HCV infection (Abe, Kurata et al. 1993), and therefore represented an ideal model to study HCV *in vivo* for over 20 years. After HCV infection, viremia as well as antiviral innate and adaptive immune responses are detectable following a mild acute hepatitis. In contrast to humans, chimpanzees rarely develop chronicity (Bassett, Brasky et al. 1998; Major, Dahari et al. 2004), and thus do not develop progressive liver disease (Walker 1997). High costs and increased ethical concerns limited the use of this animal model (National Research Council Committee on the Use of Chimpanzees in and Behavioral 2011), although it is the only well-studied model to study

protective immunity against HCV, probably leading to vaccine development (Cooper, Erickson et al. 1999).

The tree shrew (*Tupaia belangeri*), a squirrel-like mammal originally found in South-East Asia, has also been shown to be susceptible to HCV infection (Xu, Chen et al. 2007; Xie, Riezu-Boj et al. 1998) followed by chronicity and development of liver disease in some tree shrews (Amako, Tsukiyama-Kohara et al. 2010). Moreover, ethical concern and lack of tool to analyze HCV-host interactions in these animals preclude a wide usage of this model.

Rodent models represent a cheap and easy to breed model to study HCV. In general, mice and rats are not susceptible for HCV infection and need to undergo experimental modifications. The most commonly used rodent models can be divided into (i) the human liver-chimeric mouse models and (ii) the transgenic mouse models:

(i) In human liver-chimeric models, mouse livers are humanized by xenotransplantation of primary human hepatocytes or hepatoma cell lines resulting in a subsequent susceptibility to HCV infection. To successfully xenograft human hepatocytes into mouse livers, the xenograft recipients require specific genetic defects leading to death of the original mouse hepatocyte and to dysfunctionality of the mouse immune system. Currently, two major types of mice are used: first, mice overexpressing the urokinase plasminogen activator (uPA) gene under albumin promoter control were crossed with mice that suffer from a severe combined immunodeficiency syndrome (SCID) resulting in uPA-SCID mice; once xenografted with human hepatocytes, these mice are efficiently infected with HCV followed by chronic infection (Mercer, Schiller et al. 2001) and led to achievements concerning preclinical studies of antiviral compounds (Mailly, Xiao et al. 2015) and understanding of steps in the viral lifecycle (e.g. composition of LVPs, role of HCV-specific antibodies or viral entry) *in vivo* (Lindenbach, Meuleman et al. 2006; Vanwolleghem, Bukh et al. 2008; Lacek, Vercauteren et al. 2012; Meuleman, Catanese et al. 2012).

Second, mice that have fumarylacetoacetate hydrolase (*Fah*^{-/-}) deficiency accumulate hepatotoxic metabolites consequently leading to liver failure (Grompe, al-Dhalimy et al. 1993). These *Fah*^{-/-} mice were crossed with mice suffering from severe immune system dysfunctionality (*Rag2*^{-/-} *IL2Rγ*^{null}) resulting in FRG mice. The liver degeneration resulting from depletion of *Fah* can be prevented by a drug, namely NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)1,3-cyclohexanedione)). Hence, this model provides an advantage over uPA-SCID mice: the timing of human hepatocyte transplantation can be anticipated by removing NTBC (Azuma, Paulk et al. 2007; Bissig, Le et al. 2007).

(ii) In contrast to human liver-chimeric mouse model, where mice show severe immune defects, the genetically humanized mouse models serve basically to study adaptive immune responses and

immuno-evasion mechanisms. The mouse livers in these model systems are provided with all human host factors required to enable (parts of) the HCV replication cycle in mouse hepatocytes. Expression of SR-BI, OCLN, CLDN1 and CD81 under an albumin promoter enables to detect HCV entry in mouse livers (see p.7); of note, in line with results from *in vitro* experiments (see p.7) expression of human OCLN and CD81 is the minimum requirement enabling the study of viral entry in mouse livers (Dorner, Horwitz et al. 2011). Additionally, knockout of STAT1, IRF1, IRF7 and IFNAR1, all important players in innate immunity, results in low-level HCV RNA replication and production of infectious virus in mice (Dorner, Horwitz et al. 2013).

1.4 HCV treatment

As we now deep dived into the HCV molecular biology to which knowledge has markedly advanced, molecular mechanisms of disease progression and vaccine development still needs to be figured out. By thinking about viral infection, the aim of every viral therapy is eradication of virus, known in hepatitis C clinical context as sustained virologic response (SVR) which is defined as undetectable HCV RNA levels over 12 to 24 weeks after end of therapy.

Throwback to the 80's and 90's, the only available therapy for HCV infection was based on interferon (INF). INF- α exerts its antiviral effect by inducing INF-stimulated genes that in turn inhibit HCV replication. Until 1998, three INF- α injections a week for up to 48 weeks were the therapy of choice, unfortunately resulting in cure of only one-fifth of infected patients (Carithers and Emerson 1997; Hoofnagle and di Bisceglie 1997). This application was improved by injection of INF- α combined with orally taken ribavirin up to 24 to 48 weeks, an antiviral medication which could increase the antiviral effect of INF- α resulting in around 35-45% of cure in infected patients (McHutchison, Gordon et al. 1998). The addition of polyethylene glycol to INF- α (PEG-INF- α) gave rise to increased half-life of the drug and in combination with ribavirin an increased effectivity (Manns, McHutchison et al. 2001) was achieved replacing the standard INF- α for more than ten years. Of note, HCV-infected patients responded differently to the PEG-INF- α /ribavirin therapy depending on their HCV genotype: while a SVR of around 50% was achieved for patients infected with HCV genotype 1, up to 80% of SVR was shown for patients infected with genotypes 2, 3, 5 and 6 (Antaki, Craxi et al. 2010). Nevertheless, the INF-based therapy had many side-effects including flu-like symptoms, gastritis or even worse depression (Manns, Wedemeyer et al. 2006).

The achievement of uncovering key viral proteins involved in the HCV replication cycle led to the development of direct-acting antivirals (DAAs) which are specifically targeting nonstructural proteins of HCV. DAAs can be classified into four main groups: NS3/NS4A protease inhibitors,

NS5B nucleoside and non-nucleoside polymerase inhibitors and NS5A inhibitors. In 2011, a NS3/4A protease inhibitor was combined with the PEG-INF- α /ribavirin increasing SVRs for genotype 1. Nevertheless, this triple therapy was only available for genotype 1-infected patients combined with severe side-effects (Bacon, Gordon et al. 2011; Jacobson, McHutchison et al. 2011; Zeuzem, Andreone et al. 2011). In 2014-2015, new interferon-free DAA regimen, including sofosbuvir (NS5B inhibitor) and daclatasvir or ledipasvir (NS5A inhibitor), became much more efficient (SVR around 90%) with the big plus of only little side-effects (Afdhal, Zeuzem et al. 2014; Afdhal, Reddy et al. 2014; Sulkowski, Gardiner et al. 2014). The combination of different DAAs - that are now the current standard of care - lead to highly improved SVR rates and shortened therapy duration; moreover, defined DAA combinations are efficient for all HCV genotypes resulting in SVR rates of 95% (reviewed in (Asselah, Boyer et al. 2016; Li and De Clercq 2017)). Currently recommended DAAs are: dasabuvir (DSV), elbasvir (EBR), glecaprevir (GLE), grazoprevir (GZR), ledipasvir (LDV), ombitasvir (OBV), pibrentasvir (PIB), paritaprevir (PTV), ritonavir (r), sofosbuvir (SOF), velpatasvir (VEL) and voxilaprevir (VOX). Among all these recommended DAAs, the indication is depended on the HCV genotype, the severity of liver disease as well as prior therapy. Combinations of DAAs are very efficient, even if combinations of 2 regimens is preferred to triple combination in order to avoid drug-drug interactions and severe side-effects (see Table 1) (EASL 2018).

Genotype	Pangenotypic regimens			Genotype-specific regimens		
	SOF/ VEL	GLE/PIB	SOF/ VEL/ VOX	SOF/ LDV	GZR/ EBR	OBV/ PTV/r + DSV
Genotype 1a	Yes	Yes	No*	Yes*	Yes ^b	No
Genotype 1b	Yes	Yes	No*	Yes	Yes	Yes
Genotype 2	Yes	Yes	No*	No	No	No
Genotype 3	Yes ^c	Yes	Yes ^d	No	No	No
Genotype 4	Yes	Yes	No*	Yes*	Yes ^e	No
Genotype 5	Yes	Yes	No*	Yes*	No	No
Genotype 6	Yes	Yes	No*	Yes*	No	No

Table 1. Combinational DAA treatment.

Combination of DAA treatment. Recommendation for treated and non-treated patients without or with compensated cirrhosis (a-e). *Triple combination. DSV: dasabuvir, EBR: elbasvir, GLE: glecaprevir, GZR: grazoprevir, LDV: ledipasvir, OBV: ombitasvir, PIB: pibrentasvir, PTV: paritaprevir, r: ritonavir, SOF: sofosbuvir, VEL: velpatasvir, VOX: voxilaprevir (EASL 2018).

Even if may appear that with the achievement of DAAs HCV research could be disregarded, there are still some important challenges that remain (reviewed in Bartenschlager, Baumert et al. 2018). First, DAAs are only available to around 10% of infected patients (Edlin 2016) and the high costs of DAA-based therapies limit the access to a minority (Iyengar, Tay-Teo et al. 2016). Second, there are some difficult-to-treat patient populations existing with decreased SVR in response to DAAs, even

more in the presence of advanced liver disease progression. These patients are less able to clear the virus suggesting that there is still need to investigate the fine tuning of optimal DAA combination, treatment duration and dosages for difficult patient populations (Hezode, Fontaine et al. 2013; Ferenci 2015). Third and in line with the second limitation, the risk to develop HCC can be reduced after SVR, but HCC can occur after years of viral clearance suggesting that there is a “point-of-no-return” when virus-induced liver disease came to the point where HCC will develop even in the absence of HCV (Baumert, Juhling et al. 2017). Fourth, a minority of patients can develop resistance to DAA due to the error-prone HCV RNA polymerase which creates a pool of genetic variants in each patient leading to viral polymorphism in DAA-targeted regions resulting in resistance to DAAs (Esposito, Trinks et al. 2016; Pawlotsky 2016).

In addition to DAAs, the standard of care for chronic hepatitis C, alternative strategies have been developed. When reminiscing about the HCV replication cycle, several host factors play a crucial role in different steps. When reminiscing about the HCV replication cycle, several host factors play a crucial role in different steps.

Indeed, host-targeting agents (HTAs) appeared to be of therapeutic interest. Monoclonal antibodies specifically targeting CD81 (Fofana, Xiao et al. 2013), CLDN1 (Mailly, Xiao et al. 2015; Fofana, Krieger et al. 2010) or SR-BI (Meuleman, Catanese et al. 2012; Zahid, Turek et al. 2013) were developed and could be shown to efficiently inhibit viral entry *in vitro* and *in vivo* in liver chimeric mice. Additionally, small molecule inhibitors of SR-BI, ITX 5061 and ITX 7650, could efficiently block HCVcc and HCVpp infection of PHH or human hepatoma cell lines (Syder, Lee et al. 2011). ITX 5061 has been evaluated in phase 1 clinical trials (ClinicalTrials.gov identifier: NCT01165359 and NCT01292824). Moreover, a monoclonal CLDN1-specific antibody was shown to cure chronic HCV infection in the uPA-SCID chimeric mouse model (Mailly, Xiao et al. 2015; Colpitts, Tawar et al. 2018). Of note, synergy between host-targeting entry inhibitors combined with DAA-based treatment could be demonstrated (Xiao, Fofana et al. 2015; Paciello, Urbanowicz et al. 2016).

Another important host factor for the HCV life cycle is liver-enriched miR-122 that has been shown to play a crucial role in the stabilization of HCV RNA and consequently in HCV replication and translation (Jopling, Yi et al. 2005). miR-122 antagonists significantly decrease HCV replication (Jopling, Yi et al. 2005; Jopling, Schutz et al. 2008) and miravirsin and RG-101 demonstrated efficacy against HCV in clinical trials (van der Ree, de Vree et al. 2017; Stelma, van der Ree et al. 2017). In fact, these inhibitors can be regarded as pan-genotypic antivirals due to the conserved miR-122 binding sites across HCV genotypes (Li, Gottwein et al. 2011; van der Ree, de Vree et al. 2017). Cyclophilin A (CypA) inhibitors, namely alisporivir or DEB025, could efficiently block CypA interaction with NS5A leading to inhibition of HCV replication and additionally, these inhibitors are able to rehabilitate the innate immune response against HCV (Daito, Watashi et al. 2014; Hopkins,

Bobardt et al. 2012; Naoumov 2014). Phase 3 clinical trials that examined a triple therapy with alisporivir (DEB025), PEG-INF and RBV have been completed (ClinicalTrials.gov identifier: NCT01446250, NCT01318694).

In fact, the given examples represent only a small overview of HTAs that have been studied for their ability to improve antiviral treatment, several other host factors involved in the viral life cycle are at different stages of preclinical or clinical development (reviewed in Crouchet, Wrensch et al. 2018; Zeisel, Crouchet et al. 2015; Zeisel, Lupberger et al. 2013 (see Figure 10).

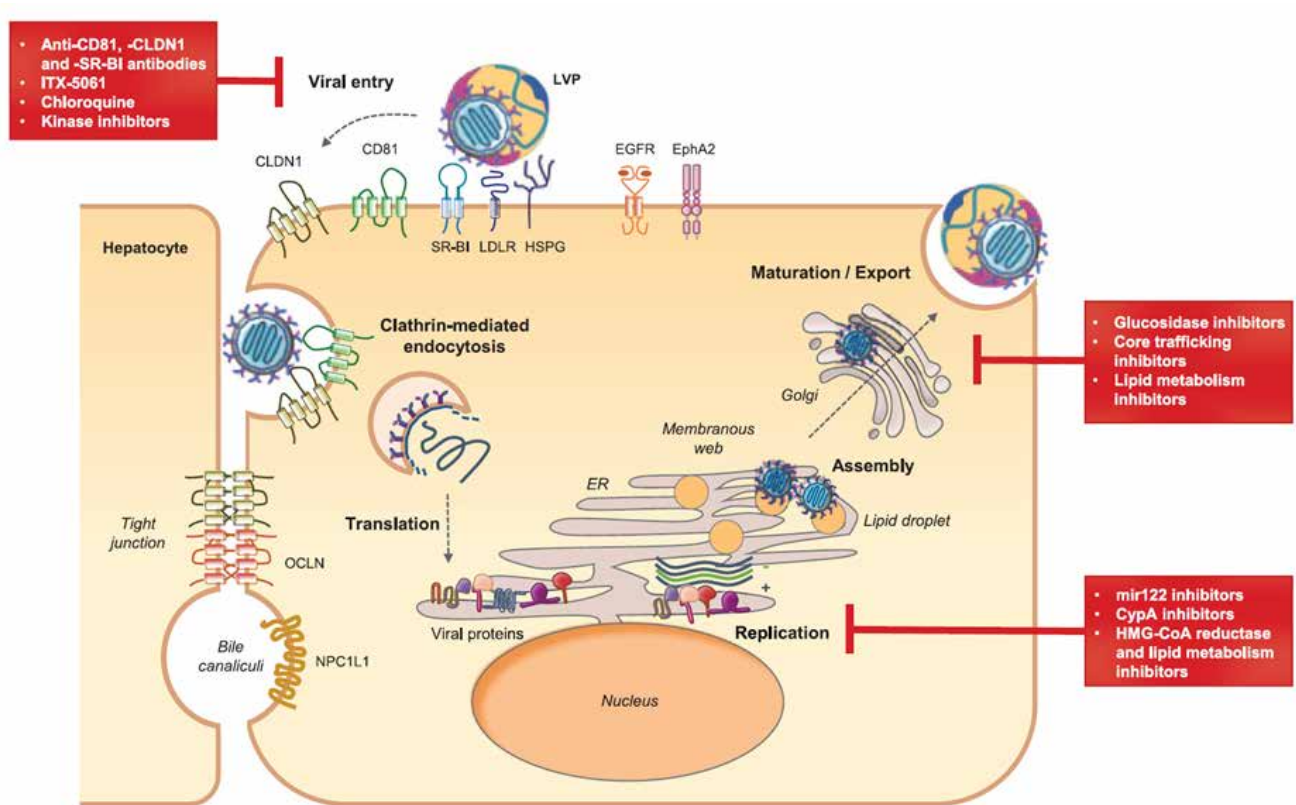


Figure 10. Host-targeting agents (HTAs) at different steps of the HCV replication cycle (Crouchet, Wrensch et al. 2018).

Taken together, HCV infection can now be regarded as curable disease, although some limitations and challenges still exist. Since HCC can develop even after successful eradication of HCV, a current question is what kind of molecular imprinting is left by HCV in the host genome that drives carcinogenesis even after SVR in patients (see HEPATITIS C – An Introduction).

II. PROTEIN GLYCOSYLATION

2.1 Post-translation modifications of HCV proteins

Post-translational modifications (PTMs) are as the name implies covalent and enzymatic modifications of proteins after completed translation and needed for protein maturation and activation. Different PTMs are known, some occur more often than others: e. g. phosphorylation, glycosylation, ubiquitination, sumoylation, nitrosylation, methylation, acetylation and lipidation; proteolysis can also be regarded as a PTM. In general, proteins are either directly modified after translation for stabilization, mediation of correct folding or recruitment of the nascent protein to their cell compartment of need; or PTMs occur after folding and localization to activate or inactivate proteins (cell signaling) contributing to the biological activity of the protein. Of note, both possibilities how and when PTMs takes place do not exclude each other but are often combined in a stepwise mechanism. While protein PTMs can be reversible, proteolysis processes are permanent.

Since HCV replication cycle is depended on the interaction with several host cell factors, it is not surprising that HCV proteins undergo PTMs for protein function and regulation as well. One can summarize a few key modifications that occur during the viral replication cycle: the proteolytic cleavage of the polyprotein, glycosylation of the HCV envelope glycoproteins E1 and E2, as well as some PTMs of the nonstructural proteins. Among the latter, phosphorylation of NS5A has been well-described: NS5A is found in a hypo-or hyper-phosphorylated form which is important for regulation of its function (Chong, Hsu et al. 2016; Appel, Pietschmann et al. 2005). Indeed, it could be shown that knockout of kinases important for this phosphorylation consequently led to reduced NS5A phosphorylation resulting in lower HCV RNA levels (Lee, Chen et al. 2016), suggesting that the phosphorylation status of NS5A plays a crucial role for HCV replication (Appel, Pietschmann et al. 2005). It is suggested that PTMs are important for the location of HCV proteins, however, questions about the function and biological relevance of these PTMs still need to be answered (reviewed in Hundt, Li et al. 2013).

The focus here are glycosylation and to a small extent phosphorylation, the most common PTMs, that could compete for the same target protein/residue.

Phosphorylation is defined in simple words as the dynamic and reversible addition or removal of phosphate residues to tyrosine, serine and threonine residues by kinases and phosphatases, respectively, resulting in activation or inactivation of proteins. Glycosylation, on the other hand, is the addition of sugar-moieties to proteins, ranging from simple monosaccharide modifications to highly complexed branched polysaccharide changes. The sugar-moieties are added either to amino

group of asparagine (N-linked) in the ER or to the hydroxyl group of serine/threonine (O-linked) of nuclear and cytoplasmic proteins (reviewed in Levine and Walker 2016) (see Figure 11).

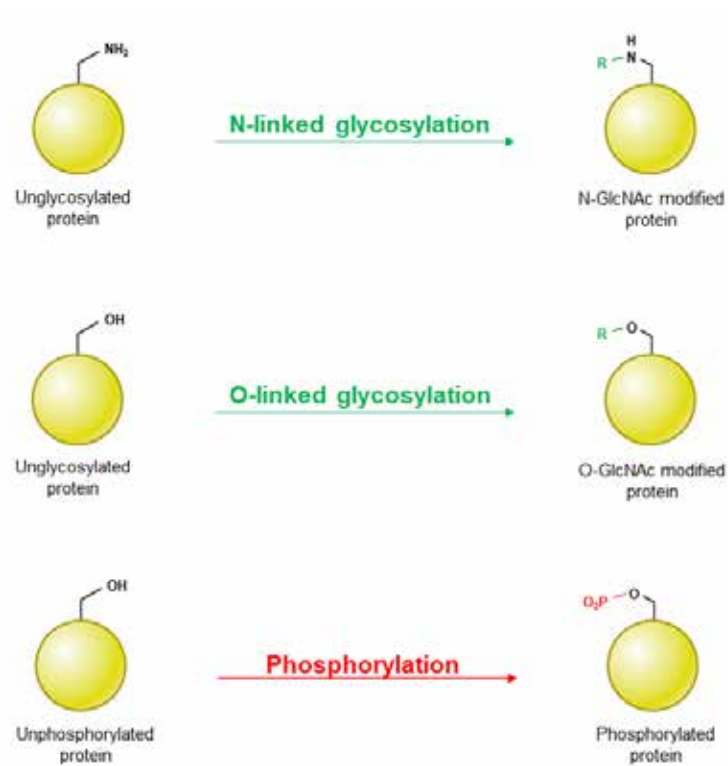


Figure 11. Basic principle of N-and O-linked glycosylation, and phosphorylation. -R: sugar residues, $-\text{PO}_3$: phosphate residues (adapted from Levine and Walker 2016).

When looking retrospectively at section 1.2.2, it was mentioned that the big challenges of HCV vaccine development are the genetic heterogeneity of the virus, its ability to rapidly mutate and its close interaction with the host lipid metabolism leading to escape from protective immune responses. However, another important key challenge regarding vaccine design is high glycosylation of the ectodomains E1 and E2 with N-glycans resulting in a glycan shield and representing one-third of the heterodimer mass (Goffard, Callens et al. 2005) (reviewed in Lavie, Hanouille et al. 2018). This shield enables the virus to avoid recognition by the humoral immune response and prevents neutralization by HCV-specific antibodies, since glycans are host-derived and thus recognized as self-patterns.

There are N-glycosylation sites that are conserved among the seven genotypes but there are some N-glycosylation sites present only in distinct genotypes, however all of the N-glycosylation sites have been confirmed to be engaged by glycans (Goffard, Callens et al. 2005). Four N-glycosylation sites can be found for E1 at positions 196 (E1N1), 209 (E1N2), 234 (E1N3), and 305 (E1N4) on the reference strain H77 (gt 1a), additionally N-glycosylation sites at position 250 (gt 1b and 6) and position 299 (gt 2b) could be found (Helle, Goffard et al. 2007). Whereas E2 contains nine conserved N-glycosylation sites at position 417 (E2N1), 423 (E2N2), 430 (E2N3), 448 (E2N4), 532 (E2N6),

556 (E2N8), 576 (E2N9), 623 (E2N10), and 645 (E2N11); two additional glycosylation sites can be detected at position 476 (E2N5) (except for gt 1b) and position 540 (E2N7) (gt 3 and 6) (see Figure 12).

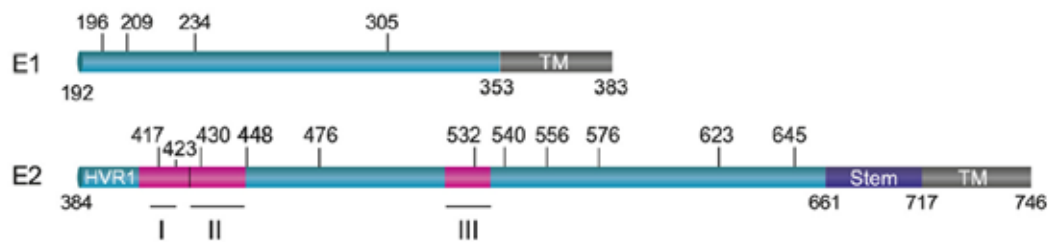


Figure 12. Schematic representation of N-glycosylation sites of E1 and E2.

Numbers and vertical bars correspond to position of glycosylation sites with the reference strain H77 (gt 1a). TM: Transmembrane domain, HVR1: Hypervariable region 1. I (412-423), II (427-446) and III (523-535) represent the three major neutralizing epitopes on E2 (Lavie, Hanouille et al. 2018).

There are three major N-glycans on HCV E1E2 based on N-acetylglucosamines and associated with: (i) several mannose residues (high mannose glycans), (ii) galactose, and sometimes sialic acid and fucose (complex oligosaccharides), and (iii) galactose, mannose and probably sialic acid (hybrid glycans). In fact, the latter two are produced during the transfer of proteins through the Golgi by enzymatic addition or removal of sugar residues mediated by glycosidases and glycosyl transferases. Indeed, after assembly in the ER and during transport along the VLDL secretory pathway, HCV glycoproteins are modified by the two latter enzymes. Differences in their glycosylation patterns could be observed between HCVpp and HCVcc resulting from the different assembly processes of HCVpp and HCVcc in the post-Golgi compartment or in an ER-derived compartment, respectively: while E1E2 derived from HCVpp contain a majority of complex-type glycans, HCVcc-derived E1E2 display high-mannose and complex N-linked glycans (Vieyres, Thomas et al. 2010) suggesting a diverse glycan processing and a different organization of proteins on the surface of HCVpp or HCVcc (reviewed in Lavie, Hanouille et al. 2018). As a consequence, HCVpp and HCVcc act differently during their entry processes (Johansson, Voisset et al. 2007; Kapadia, Barth et al. 2007; Russell, Kawaguchi et al. 2009; Albecka, Montserret et al. 2011; Wasilewski, Ray et al. 2016).

Two benefits of N-glycans on HCV glycoproteins E1 and E2 are obvious: First, N-glycans, normally involved in protein folding (Helenius and Aebi 2001), can affect the binding affinity of E1E2 for receptors. Second, N-glycans on E1E2 are effective in shielding E1 and E2 from the humoral immune response and thus prevent the virus from being neutralized by HCV-specific antibodies, consequently to a non-effective immune response (de Jong, Dorner et al. 2014). In fact, HCV E2 is the main target of neutralizing antibodies (reviewed in Ball, Tarr et al. 2014). When looking again at Figure 12, the three major neutralizing epitopes of E2 (mentioned as I-III) are N-glycosylated resulting in an

effective shield of those epitopes and an evasion of the host immune response. Additionally, one group could observe escape mutants from an E2-specific antibody (AP33) displaying a glycan shift (Pantua, Diao et al. 2013) suggesting that the N-glycosylation of HCV glycoproteins and the evasion of the host immune response is not a fixed but rather dynamic process.

Apart from N-glycosylation, O-glycosylation sites have been predicted on the E2 envelope protein as well (Bandiera and Zeisel, unpublished data and (reviewed in Lavie, Hanouille et al. 2018), however, they have not been well-described.

2.2 *O*-linked *N*-acetylglucosaminylation (*O*-GlcNAcylation)

O-linked *N*-acetylglucosaminylation (*O*-GlcNAcylation) is a dynamic and reversible PTM that mainly takes place in the cytoplasmic, mitochondrial, and nuclear compartments of the cell (Issad, Masson et al. 2010), and appears to show similar features to phosphorylation, since the modification sites for both are serine and threonine residues. Indeed, an extensive crosstalk between *O*-GlcNAcylation and phosphorylation could be observed, either by competing for the same serine or threonine residue or by *O*-GlcNAcylation influencing phosphorylation of adjacent residues (Leney, El Atmioui et al. 2017) (see Figure 13).

The substrate of *O*-GlcNAcylation, Uridine-diphosphate *N*-acetylglucosamine (UDP-GlcNAc) is synthesized as an end product of the hexosamine biosynthetic pathway (HBP); an alternative pathway within the glucose metabolic pathway that converts up to 2%-5% of cellular glucose into UDP-GlcNAc involving several enzymes (reviewed in Buse 2006). UDP-GlcNAc serves as the donor for the addition of GlcNAc to serine and threonine residues mediated by *O*-linked *N*-acetylglucosamine transferase (OGT), whereas the removal of *O*-GlcNAc is mediated by *O*-GlcNAc hydrolase (OGA) (reviewed in Levine and Walker 2016). Of note, OGT and its counterpart OGA are the unique enzymes regulating *O*-GlcNAc levels within cells (see Figure 13).

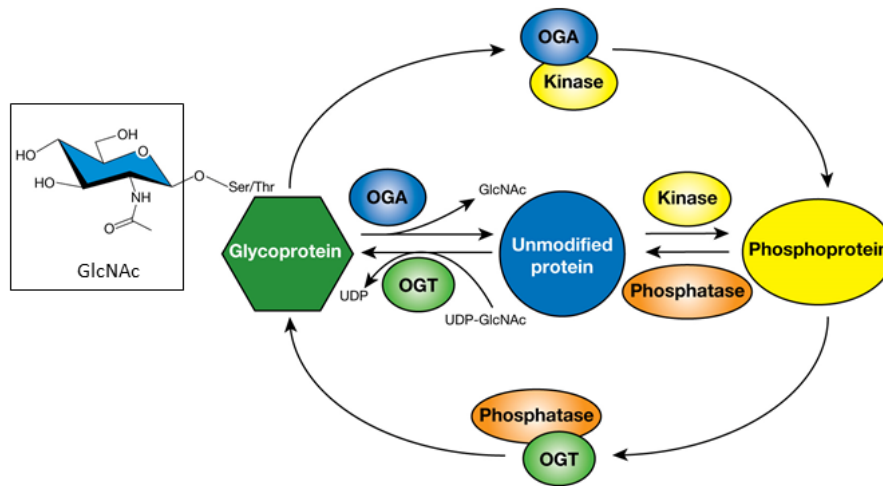


Figure 13. *O*-GlcNAcylation versus phosphorylation.

Dynamic addition and removal of *O*-GlcNAc mediated solely by OGT and its counterpart OGA. For some target proteins, *O*-GlcNAc and phosphorylation compete for serine and threonine residues (adapted from Zachara, Akimoto et al. 2015).

OGT is found in three isoforms in mammals (i) nucleocytoplasmic (ncOGT, 116 kDa), (ii) mitochondrial (mOGT, 103 kDa), and (iii) short (sOGT, 78 kDa), all containing a N-terminal domain consistent of tetratricopeptide repeat (TPR) motifs which are important to recognize the different protein substrates, and a C-terminal domain implying the glycosyltransferase activity (reviewed in Joiner, Li et al. 2019; Liu, Dai et al. 2015) (see Figure 14). In addition to its role in *O*-GlcNAcylation of nuclear, cytoplasmic and mitochondrial proteins, OGT has scaffold function and promotes the stable binding of proteins in multiprotein complexes (Hart, Housley et al. 2007).

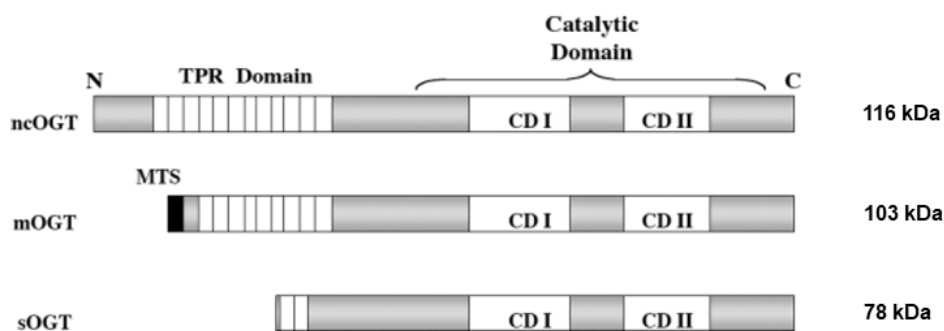


Figure 14. The three isoforms of OGT.

N-terminal domain contains tetratricopeptide repeat (TPR) motifs which are important for the recognition of protein substrates, while the C-terminal domain consists of the glycosyltransferase activity. Nucleocytoplasmic OGT (ncOGT, 116 kDa), mitochondrial OGT (mOGT, 103 kDa) and short OGT (sOGT, 78 kDa) (adapted from Lazarus, Love et al. 2006).

OGA, on the other hand, is expressed as two isoforms (i) long, nucleocytoplasmic (OGA-L, 102 kDa) and (ii) short, nuclear (OGA-S, 76 kDa) (reviewed in Liu, Dai et al. 2015).

Since knockout of OGT in mammals (Shafi, Iyer et al. 2000) and OGA in mice (Yang, Song et al. 2012) is lethal on a developmental or single cell level, respectively, the design of effective inhibitors raised the ability to study *O*-GlcNAcylation *in vivo*. Gloster *et al.* generated metabolic inhibitors of glycosyltransferases within cells using sugar analogues. After its uptake, Ac-5SGlcNAc -one of those inhibitors- is converted to UDP-5SGlcNAc which inhibits activity of OGT by direct binding leading to decreased levels of *O*-GlcNAc in cells (Gloster, Zandberg et al. 2011). Other small molecules to inhibit OGT have been reported with controversial observations: (i) alloxan that completely blocks activity of OGT *in vitro* but is not specific and inhibits other critical enzymes (Lee, Alborn et al. 2006), (ii) benzoxazolinone also inhibits OGT by forming a carbonyl crosslink in the OGT active site which is an irreversible process (Jiang, Lazarus et al. 2011), (iii) OSMI-1, which is a cell-permeable molecule that is able to inhibit OGT in several mammalian cell lines (Ortiz-Meoz, Jiang et al. 2015), however, high cytotoxicity was observed in human hepatoma cells (Herzog and Zeisel, unpublished data) or (iv) ST045849, which leads to decrease of total *O*-GlcNAcylation in prostate cancer cells (Itkonen, Gorad et al. 2016), while in human hepatoma cells increased levels of *O*-GlcNAcylation have been observed (Herzog and Zeisel, unpublished data). On the other hand, *O*-GlcNAcylation in cells is increased by efficient inhibition of OGA via Thiamet G (Yuzwa, Macauley et al. 2008) and GlcNAcstatin C (Dorfmueller, Borodkin et al. 2006) *in vitro* (Mariappa, Sauert et al. 2011) as well as *in vivo* (Yuzwa, Shan et al. 2012). Recently, a metabolic precursor inhibitor that acts as an analogue of N-acetylglucosamine, termed 5SGlcNH₆, was shown to decrease *O*-GlcNAc levels in time- and dose-dependent manner in various tissues. Moreover, a correlation between decreased *O*-GlcNAc levels and lower levels of transcription factor Sp1 and satiety-induced hormone leptin could be demonstrated *in vitro* in adipocytes as well as *in vivo* in mice proposing an association between decreased *O*-GlcNAc levels and nutrient sensing in peripheral tissues of mammals (Liu, Zandberg et al. 2018).

O-GlcNAcylated proteins of different functional classes have been detected, including transcription factors, metabolic enzymes and signaling proteins (see Figure 15) highlighting a crucial role of *O*-GlcNAcylation in many biological processes as transcription, translation, metabolism, signal transduction and autophagy (Butkinaree, Park et al. 2010; Ferron, Denis et al. 2018). Additionally, proteins with intrinsic disorders can be non-specifically modified by OGT as well (e.g. tau and nuclear pore proteins) (reviewed in Yang and Qian 2017).

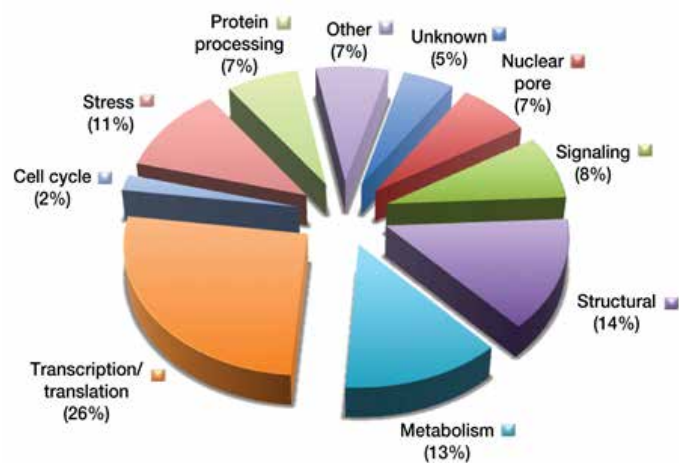


Figure 15. Functional distribution of identified *O*-GlcNAcylated proteins (adapted from Zachara, Akimoto et al. 2015).

2.3 OGT and *O*-GlcNAcylation in disease and cancer- Current knowledge for liver disease and HCC

O-GlcNAcylation plays a crucial role in many different biological processes and is solely performed by OGT and OGA; due to this fact, it is obvious that deregulated *O*-GlcNAcylation has been associated with a variety of human diseases ranging from diabetes and neurodegenerative and cardiovascular diseases to cancer (reviewed in Banerjee, Lagerlof et al. 2016; Pinho, Verde et al. 2018; de Queiroz, Carvalho et al. 2014; Fardini, Dehennaut et al. 2013). However, detailed knowledge how and which *O*-GlcNAcylation contributes to these diseases remains incomplete.

In fact, the present thesis is based on HCV and virus-host interactions. Since it is known that HCV infection, even after SVR, can lead to progressive liver disease and HCC, I will focus here, for clarity reasons, on the current knowledge about *O*-GlcNAcylation and its contribution to cancer development, especially for HCC development.

Basically, cancer cells are characterized by uncontrolled proliferation and show a specific metabolic phenotype in which ATP is primarily produced through anaerobic glycolysis rather than through oxidative phosphorylation. The metabolic shift is widely known as “Warburg effect”, after the discovery made by Otto Warburg resulting in an up-regulation of glucose uptake and consequently in increased flux of metabolites through the HBP (Warburg 1956). This phenomenon of metabolic shift is characterized through an increased *O*-GlcNAcylation, observed in a wide range of cancers, resulting in an altered glycosylation profile leading to deregulated signaling and transcriptional pathways (reviewed in (Ferrer, Sodi et al. 2016; Liberti and Locasale 2016) (see Table 2).

Tumorigenesis, invasion and metastasis of breast, lung and colon cancer is enhanced through aberrant *O*-GlcNAcylation (Caldwell, Jackson et al. 2010; Gu, Mi et al. 2010; Mi, Gu et al. 2011; Ferrer, Lu et al. 2017). It has been demonstrated that aberrant flux through HBP (reviewed in Bond and Hanover

2015) resulted in alteration of cell surface *O*-Glycans (Alisson-Silva, Freire-de-Lima et al. 2013) as well as *N*-Glycans (Dennis, Lau et al. 2009), however, the focus here is on *O*-GlcNAcylation. One outcome of the deregulated signaling pathways is the gain of function of oncogenes and a loss of function of tumor suppressors. Indeed, it could be demonstrated that *O*-GlcNAcylation is able to modify several tumor-associated proteins as for example Nuclear factor κ B (NF- κ B), c-Myc, β -catenin, p53 and Ras (reviewed in de Queiroz, Carvalho et al. 2014; Nie and Yi 2019). Additionally, it has been demonstrated that several HBP genes were overexpressed in human prostate cancer as well (Itkonen, Minner et al. 2013).

HCC is the most frequently occurring primary liver malignancy worldwide, in fact liver transplantation (LT) is the only treatment of choice available for patients with early stage of HCC (reviewed in El-Serag and Davila 2011; El-Serag and Rudolph 2007). Nevertheless, tumor recurrence following LT remains so far an unsolved problem preventing patients from long-term survival. The addressed question is which are the molecular mechanism of tumor recurrence after LT? Zhu et al. tried to elucidate this question by studying *O*-GlcNAcylation levels in HCC tissues of patients with different etiologies (Zhu, Zhou et al. 2012). Indeed, it has been shown that *O*-GlcNAcylation levels of HCC tissues was significantly increased as compared to healthy liver tissue; moreover, HCC tissue of those patients that suffered from tumor recurrence after LT had significantly enhanced *O*-GlcNAcylation levels (Zhu, Zhou et al. 2012). While OGT expression could not be correlated with a prognosis for HCC recurrence after LT, low OGA expression in tumors has been elevated with an increased risk of tumor recurrence after LT (Zhu, Zhou et al. 2012).

NF- κ B, a transcription factor known to play a crucial role in cancer-related processes as cell proliferation, apoptosis and metastasis (reviewed in Patel, Horgan et al. 2018) displays two *O*-GlcNAcylation sites at its subunit p65, at threonine 322 and 352. The latter glycosylation site is responsible for an increased transcriptional activity (Yang, Park et al. 2008). Interestingly, another group observed a tumor-promoting role of OGT in fatty liver-associated HCC by regulating lipid metabolism through increasing palmitic acid leading to ER stress and ER-related NF- κ B signaling pathways proposing an oncogenic role for OGT in fatty-liver associated HCC (Xu, Zhang et al. 2017). Most recently, another group could reveal an increase of total *O*-GlcNAcylation and an increase of OGT protein expression in HCC (Cao, Duan et al. 2019). Moreover, it could be shown that eukaryotic initiation factor 4E (eIF4E), a key translation factor of protein synthesis, is *O*-GlcNAcylated by OGT leading to stability of protein and protection against proteasomal degradation; as a result, it is proposed that *O*-GlcNAcylation contributes to a stem-like cell potential of hepatoma cells (Cao, Duan et al. 2019).

Taken together, O-GlcNAcylation has a crucial role for reprogramming metabolic networks of cancer cells and in turn in promoting tumor growth and carcinogenesis. Table 2 gives an overview of deregulated O-GlcNAcylation and aberrant OGT/OGA protein expression in different kind of cancers.

Tumor type	O-GlcNAc/OGT/OGA	References
Liver cancer	↑/↑/↓	Cao, Duan et al. 2019; Xu, Zhang et al. 2017; Zhu, Zhou et al. 2012
Breast cancer	↑/↑/↓	Liu, Huang et al. 2017; Ferrer, Lu et al. 2017; Caldwell, Jackson et al. 2010; Gu, Mi et al. 2010; Barkovskaya, Seip et al. 2019
Prostate cancer	↑/↑/NI	Kamigaito, Okaneya et al. 2014; Itkonen, Minner et al. 2013; Itkonen, Gorad et al. 2016; Lynch, Ferrer et al. 2012; Itkonen, Urbanucci et al. 2019
Colon cancer	↑/↑/↓	Yehezkel, Cohen et al. 2012; Mi, Gu et al. 2011; Phueaouan, Chaiyawat et al. 2013; Biwi, Clarisse et al. 2019; Fuentes-Garcia, Castaneda-Patlan et al. 2019
Bladder cancer	NI/↑/↓	Wang, Chen et al. 2018; Rozanski, Krzeslak et al. 2012
Leukemia	↑/NI/NI	Shi, Tomic et al. 2010
Endometrial cancer	NI/↑/↑	Krzeslak, Wojcik-Krowiranda et al. 2012
Pancreatic cancer	↑/↑/↓	Ma, Vocadlo et al. 2013; Sharma, Gupta et al. 2019
Lung cancer	↑/↑/ND	Mi, Gu et al. 2011; Szymura, Zaemes et al. 2019; Lin, Lin et al. 2018
Thyroid cancer	↓/NI/↑	Krzeslak, Jozwiak et al. 2011; Zhang, Wang et al. 2015; Cheng, Li et al. 2016

Table 2. O-GlcNAc dynamics in different types of cancer.
 ↑: increase; ↓: decrease; ND: No difference; NI: Not identified.

Even if an increased global O-GlcNAcylation and increased OGT protein expression could be shown to contribute to cancer development, downregulation of O-GlcNAcylation could be demonstrated to drive tumorigenesis as well (e.g. thyroid cancer). Overall, one can assume that uncovering key O-GlcNAcylated proteins and deregulated OGT/OGA expression could reveal novel biomarkers for

early detection of cancer, and especially for HCC these potential markers could help to predict patient risk of recurrence and could be potential targets for efficient therapy.

III. OBJECTIVES

Infection of human hepatocytes is a complex process involving both viral and host factors involved in the HCV replication cycle such as microRNAs (miRNAs). Within the past years, it has been shown that to establish chronic infection, HCV hijacks and modulates host cell-derived miRNAs that are required for the viral life cycle. Uncovering host factors hijacked by HCV contributes to a better understanding of virus-host interactions underlying the HCV life cycle and involvement in the establishment of chronic HCV infection as well as to the identification of potential targets for treatment of liver disease and prevention of HCC (Zeisel and Baumert 2017; Zeisel, Crouchet et al. 2015).

To systematically uncover human miRNAs affecting the HCV replication cycle, the laboratory had performed a genome-wide screen in human hepatoma Huh7.5.1 cells using a genomic miRNA mimic library and a two-step infection assay with a *Renilla* luciferase reporter HCV virus (JcR2a) (Herzog, Bandiera et al. 2019, figure 1A). Through this miRNA mimic screen 427 miRNAs could be identified that significantly modulated HCV infection (Herzog, Bandiera et al. 2019, figure 1C). One hundred eighty-six miRNAs out of the 427 miRNAs affected HCV entry and replication, while the remaining 309 miRNAs modified viral assembly and release (including 68 hits affecting both HCV entry/replication and assembly/release) (Figure 16).

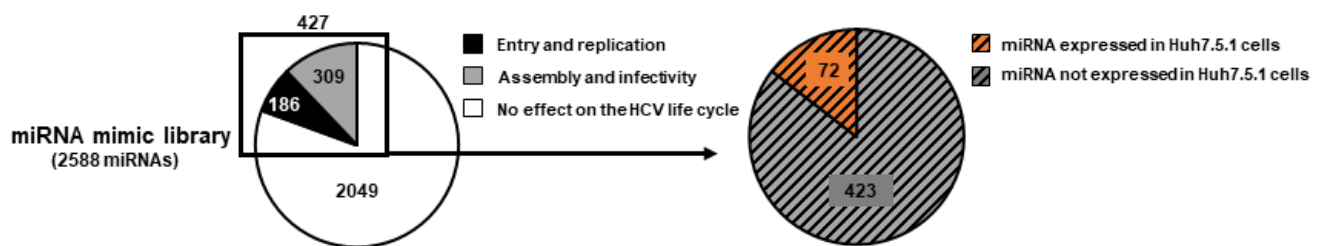


Figure 16. miRNAs involved in the HCV life cycle and expression in Huh7.5.1 cells.

Thus, it was decided to focus on miRNA hits that modulated late steps of the HCV replication cycle since these processes steps still remain incompletely understood. Among the 309 miRNAs, miR-501-3p and miR-619-3p appeared to markedly enhance HCV infection suggesting that these miRNAs may target host genes which contribute to control virus assembly and release (Herzog, Bandiera et al. 2019, figure 1D). By using a combination of computational and functional approaches to study miRNA targeting, OGT was uncovered as a common target for miR-501-3p and miR-619-3p and

demonstrated to be markedly involved in assembly and infectivity (Herzog, Bandiera et al. 2019, table 1 and figure 2).

Hence, the aim of my PhD project was to characterize the role of OGT in HCV-host interactions. The specific aims of my PhD project were i) to analyze the interplay of miR-501-3p and miR-619-3p with their predicted target OGT, ii) to unravel how OGT may contribute to HCV morphogenesis and, iii) to investigate the role of miR-501-3p and OGT in HCV-induced liver disease and HCC development.

IV. RESULTS

To systematically uncover miRNAs that could play a role in HCV infection, the team of Mirjam Zeisel and Thomas Baumert had conducted a genome-wide screen in human hepatoma Huh7.5.1 cells using a genomic miRNA mimics library and a two-step infection assay with a luciferase reporter virus (JcR2a), which allowed us to functionally assess the role of miRNAs during the early steps (viral entry/translation/replication) and the late steps (viral assembly/release/infectivity) of the HCV life cycle. This screen identified a set of miRNAs whose overexpression overall impairs HCV infection by affecting early and/or late steps of the HCV replication cycle. The analysis was focused on miRNAs that modulate late steps of the HCV life cycle, as the molecular mechanisms underlying HCV assembly/release still remain only partially understood. The screen had identified 11 miRNAs that increased and 230 miRNAs that decreased late steps of HCV infection without affecting early steps of infection. Among the miRNAs that increased HCV infection, miR-140-3p, miR-501-3p, miR-619-3p and miR-4778-5p had not been associated with HCV before. The effect of two out of these 4 miRNAs, namely miR-501-3p and miR-619-3p, on late steps of the HCV life cycle were confirmed in independent experiments. The targets of these two miRNAs were predicted using several bioinformatics tools and the list of potential targets were refined by considering only those genes that are i) expressed in Huh7.5.1 cells as assessed using a microarray analysis and ii) belonging to pathways potentially involved in infectious virus production, such as lipid metabolism and cholesterol biosynthesis, protein maturation and processing at the ER and components of the endosomal sorting complex. This led to a list of 28 candidate targets whose functional role in HCV infection was subsequently assessed using a two-step infection assay with a luciferase reporter virus (JcR2a) and siRNA directed against these 28 targets. Interestingly, only the silencing of OGT phenocopied the effect of miR-501-3p and miR-619-3p on HCV infection, suggesting that OGT modulates HCV assembly, release and/or infectivity.

During my PhD, to assess whether OGT could be indeed targeted by miR-501-3p and miR-619-3p, OGT expression was analyzed at both the mRNA and the protein levels in Huh7.5.1 cells following overexpression of miR-501-3p and miR-619-3p. We demonstrated that neither miRNA had an impact on the OGT mRNA levels (Herzog, Bandiera et al. 2019, figure 3A) but that miR-501-3p significantly decreased OGT protein expression (Herzog, Bandiera et al. 2019, figure 3B). Using a dual luciferase reporter construct, we showed that co-transfection of miR-501-3p mimic with the wild-type 3'UTR reporter (RLuc wt *OGT* 3'UTR) significantly decreased luciferase activity as compared to the empty vector while the repression was lost when the reporter with the mutated miR-501-3p binding site (RLuc mt *OGT* 3'UTR) was used (Herzog, Bandiera et al. 2019, figure 3C). These data indicate that

miR-501-3p mediates post-transcriptional regulation of OGT by decreasing its expression at the protein level.

To investigate the effect(s) of OGT on HCV assembly, release and/infectivity, we determined infectious virus titer (TCID₅₀), HCV RNA levels and the specific infectivity of HCVcc particles generated in Huh7.5.1 cells with silenced OGT. We observed a significant increase in the TCID₅₀ as well as in the specific infectivity of HCVcc generated in OGT-silenced Huh7.5.1 cells as compared to control HCVcc (Herzog, Bandiera et al. 2019, figure 4A). Furthermore, using pharmacological inhibitors to inhibit either OGT (Ac₄s-GlcNAc) or its counterpart OGA (Thiamet G), we demonstrated that O-GlcNAcylation modulates HCVcc infectivity.

By analyzing the structural and biophysical properties of HCVcc produced in OGT-silenced Huh7.5.1 cells, we demonstrated that silencing of OGT led to the production of more infectious HCVcc with higher density (Herzog, Bandiera et al. 2019, figure 5A-B) as well as higher ApoE concentrations (Herzog, Bandiera et al. 2019, figure 5C) suggesting that OGT/O-GlcNAcylation affects the biophysical properties of HCVcc. Electron microscopy (EM) analysis and counting of HCVcc particles revealed a shift towards bigger size of sucrose-cushion purified HCVcc generated in OGT-silenced Huh7.5.1 cells as compared to control HCVcc (Herzog, Bandiera et al. 2019, figure 6A-B) suggesting that OGT-silencing affects the lipidation of HCVcc.

Since the silencing of OGT promotes HCVcc infectivity, we also assessed whether HCV infection in turn had an effect on miR-501-3p and OGT expression. In Huh7.5.1 cells, HCV infection led to significant decrease of OGT mRNA as well as proteins levels, while a small but significant increase of miR-501-3p expression could be observed (Herzog, Bandiera et al. 2019, figure 7A-B and supplementary figure 1B) which may promote viral infection given the proviral and antiviral roles of miR-501-3p and O-GlcNAcylation, respectively (Herzog, Bandiera et al. 2019, figure 1C-D and 4D). In liver tissue from HCV-infected patients, HCV RNA levels were not correlated with OGT expression suggesting that in patients there is likely no direct effect of HCV on OGT expression (Herzog, Bandiera et al. 2019, figure 7C).

Given the fact that O-GlcNAcylation has been associated with a variety of cancers, we also studied OGT expression in chronic liver disease and HCC. While a trend of increased OGT expression in liver tissue from HCV-infected patients with fibrosis and inflammation could be observed (Herzog, Bandiera et al. 2019, figure 7D-E), OGT levels were markedly and significantly elevated in the tumor tissue of patients chronically infected with HCV or hepatitis B virus, and patients with alcoholic liver disease or non-alcoholic fatty liver disease as compared to non-tumor tissue (Herzog, Bandiera et al. 2019, figure 7F). These data suggest that OGT expression increases in HCC in an etiology-

independent manner. Taken together, these results suggest that OGT expression likely increased in HCV-induced liver disease and cancer through inflammation and fibrosis rather than by HCV itself.

These results have been published in Herzog*, Bandiera* et al. “*Functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as a host factor modulating hepatitis C virus morphogenesis and infectivity*” Gut 2019.

1 **A functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as**
2 **a host factor modulating hepatitis C virus morphogenesis and infectivity**

3
4 Katharina Herzog^{1,2*}, Simonetta Bandiera^{1,2*}, Sophie Pernot^{1,2}, Catherine Fauvelle^{1,2}, Frank
5 Jühling^{1,2}, Amélie Weiss^{2,3,4,5}, Anne Bull⁶, Sarah C. Durand^{1,2}, Béatrice Chane-Woon-Ming^{2,7},
6 Sébastien Pfeffer^{2,7}, Marion Mercey⁸, Hervé Lerat⁸, Jean-Christophe Meunier⁶, Wolfgang
7 Raffelsberger^{2,3,4,5}, Laurent Brino^{2,3,4,5}, Thomas F. Baumert^{1,2,9,#}, Mirjam B. Zeisel^{1,2,10,#}

8
9 ¹Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg,
10 France; ²Université de Strasbourg, Strasbourg, France; ³Institut de Génétique et de Biologie
11 Moléculaire et Cellulaire, Illkirch, France ; ⁴CNRS, UMR7104, Illkirch, France ; ⁵Inserm,
12 U1258, Illkirch, France ; ⁶Inserm U1259, Faculté de Médecine, Université François Rabelais
13 and CHRU de Tours, Tours, France ; ⁷Architecture et Réactivité de l'ARN – UPR 9002,
14 Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France; ⁸Institute for
15 Applied Biosciences, Centre de Recherche UGA - Inserm U1209 - CNRS 5309, Grenoble,
16 France ⁹Institut Hospitalo-Universitaire, Pôle Hépatodigestif, Hôpitaux Universitaires de
17 Strasbourg, Strasbourg, France; ¹⁰Inserm, U1052, CNRS UMR 5286, Centre Léon Bérard
18 (CLB), Cancer Research Center of Lyon (CRCL), Université de Lyon (UCBL), Lyon, France

19 *Authors contributed equally to this work

20
21 **Word count.** Abstract: 196 words; main manuscript: 3991 words; 54 references; 7 figures; 1
22 table; Supplementary information (including 1 Supplementary Table and 1 Supplementary
23 Figure)

24
25 **#Corresponding authors.** Dr. Mirjam B. Zeisel, Inserm U1052 – CRCL, 151 cours Albert
26 Thomas, 69424 Lyon Cedex 03, France, Phone: +33472681970, Fax: +33472681971, E-
27 mail: mirjam.zeisel@unistra.fr and Prof. Thomas F. Baumert, Inserm U1110, Institut de

1 Recherche sur les Maladies Virales et Hépatiques, 3 rue Koeberlé, 67000 Strasbourg,
2 France, Phone: +33368853703, Fax: +33368853724, Email: thomas.baumert@unistra.fr

3

4 **Financial support.** This work was supported by the European Union (INTERREG-IV-Rhin
5 Supérieur-FEDER-Hepato-Regio-Net 2012 to T.F.B. and M.B.Z., ERC-AdG-2014-671231-
6 HEPCIR, EU H2020-667273-HEPCAR to T.F.B), ANRS (2012/239 to T.F.B., M.B.Z. and
7 L.B.), ARC, Paris and Institut Hospitalo-Universitaire, Strasbourg (TheraHCC IHUARC
8 IHU201301187 to T.F.B.), the Impulsion Program of the IDEXLYON (to M.B.Z.), Ligue contre
9 le cancer (to M.B.Z.), Inserm, and University of Strasbourg. This work has been published
10 under the framework of the LABEX ANR-10-LABX-0028_HepSYS, ANR-10-LABX-36
11 NetRNA and Inserm Plan Cancer 2019-2023 and benefits from funding from the state
12 managed by the French National Research Agency as part of the Investments for the future
13 program. S.P. and K.H. were supported by PhD fellowships from the French Ministry of
14 Research and the IdEx program of the University of Strasbourg, respectively.

15

16 **Author contribution.** M.B.Z. coordinated and supervised research. K.H., S.B., S.P., C.F.,
17 A.W., L.B., J-C.M. and M.B.Z. designed experiments. K.H., S.B., S.P, C. F., A.W., A.B.,
18 S.C.D., M.M., H.L. and J-C.M. performed experiments. K.H., S.B., S.P., C. F., F.J., A.W.,
19 A.B., B.C.W.M., S.P., J-C.M., W.R., L.B., T.F.B. and M.B.Z. analyzed data. K.H., S.B., and
20 M.B.Z. wrote the paper.

21

22 **Competing interests:** The authors do not have competing interest.

23

1 **Abstract**

2

3 **Objective:** Infection of human hepatocytes by the hepatitis C virus (HCV) is a multistep
4 process involving both viral and host factors. microRNAs (miRNAs) are small non-coding
5 RNAs that post-transcriptionally regulate gene expression. Given that miRNAs were
6 indicated to regulate between 30% and 75% of all human genes, we aimed to investigate the
7 functional and regulatory role of miRNAs for the HCV life cycle.

8 **Design:** To systematically reveal human miRNAs affecting the HCV life cycle, we performed
9 a two-step functional high-throughput miRNA mimic screen in Huh7.5.1 cells infected with
10 recombinant cell culture-derived HCV. miRNA targeting was then assessed using a
11 combination of computational and functional approaches.

12 **Results:** We uncovered miR-501-3p and miR-619-3p as novel modulators of HCV
13 assembly/release. We discovered that these miRNAs regulate O-linked N-acetylglucosamine
14 (O-GlcNAc) transferase (OGT) protein expression and identified OGT and O-GlcNAcylation
15 as regulators of HCV morphogenesis and infectivity. Furthermore, increased OGT
16 expression in patient-derived liver tissue was associated with HCV-induced liver disease and
17 cancer.

18 **Conclusion:** miR-501-3p and miR-619-3p and their target OGT are previously undiscovered
19 regulatory host factors for HCV assembly and infectivity. In addition to its effect on HCV
20 morphogenesis, OGT may play a role in HCV-induced liver disease and
21 hepatocarcinogenesis.

22

Significance of this study

1

What is already known about this subject?

- w To establish chronic infection, the hepatitis C virus (HCV) hijacks cellular factors including microRNAs (miRNAs), known to post-transcriptionally regulate gene expression.
- w miRNAs may positively or negatively modulate HCV infection either by directly targeting the viral genome or indirectly by regulating virus-associated cellular pathways.

What are the new findings?

- w A functional miRNA mimic screen uncovered miR-501-3p and miR-619-3p to enhance late steps of HCV infection.
- w miR-501-3p regulates the expression of O-linked N-acetylglucosamine transferase (OGT) at the protein level.
- w Silencing of OGT expression or inhibition of O-linked N-acetylglucosaminylation (O-GlcNAcylation) leads to an increase in the infectivity and size of HCV particles.
- w OGT expression increases in patient-derived liver tissue during liver disease progression and cancer.

How might it impact on clinical practice in the foreseeable future?

- w As upregulation of OGT and increased O-GlcNAcylation of proteins have been associated with various forms of cancer, OGT may play a dual role in HCV morphogenesis as well as pathogenesis of HCV-induced liver disease and carcinogenesis.

2

3

1 **Introduction**

2 Chronic hepatitis C is a major cause of chronic liver disease and hepatocellular carcinoma
3 (HCC). Since the approval of pan-genotypic direct-acting antivirals (DAAs), it is considered a
4 curable disease in more than 90% of treated patients. Nonetheless, an estimated 71 million
5 individuals are still infected by the hepatitis C virus (HCV) and several challenges remain;
6 viral cure reduces but does not eliminate the HCC risk in patients with advanced fibrosis[1],
7 the majority of infected patients has limited access to therapy and DAA failure/viral
8 resistance has been reported in a subset of patients[2, 3]. To overcome these limitations,
9 approaches to target host factors involved in HCV infection and pathogenesis are
10 developed[4, 5]. Interestingly, defined host factors that contribute to the establishment of
11 chronic HCV infection and represent potential antiviral targets, e.g. epidermal growth factor
12 receptor[6], also play a role in liver disease pathogenesis and represent candidate targets for
13 treatment of advanced liver disease and HCC prevention[7]. Thus, uncovering host factors
14 usurped by HCV not only contributes to a better understanding of virus-host interactions
15 underlying the HCV life cycle but also to the identification of potential targets for treatment of
16 liver disease and prevention of HCC.

17 The establishment of various models to study HCV infection has shed light on the
18 molecular mechanisms that govern the HCV life cycle, which can be subdivided into early
19 steps, including viral entry, translation and replication as well as late steps, including
20 assembly and release of new virions. Each step of the HCV replication cycle relies on
21 specific virus-host interactions that involve host proteins and microRNAs (miRNAs)[5], small
22 non-coding RNAs that regulate gene expression at the post-transcriptional level. One miRNA
23 can target numerous messenger RNAs (mRNAs) by base-pairing with a complementary site
24 that is typically located within the 3' untranslated region (3'UTR) of the mRNA. Accumulating
25 evidence indicates that miRNAs participate to HCV replication by exerting pro- or antiviral
26 effects. The breakthrough discovery of the direct targeting of HCV by miR-122, the most
27 abundant miRNA in the liver, revealed the crucial role of this miRNA for HCV
28 translation/replication that contributes to progression to chronic HCV infection[8, 9]. miR-122

1 antisense oligonucleotides were subsequently developed as host-targeting antivirals[10, 11].
2 Other miRNAs can indirectly target HCV by regulating host factors that participate in antiviral
3 responses and immune surveillance[12, 13, 14]. Since up to 60% of all human protein-coding
4 genes were reported to be under miRNA-mediated regulation and miRNAs are involved in
5 basically every biological process, we hypothesized that miRNAs provide a tool for loss-of-
6 function approaches to uncover novel HCV host factors. We performed genome-wide high-
7 throughput modulation of the human miRNome and analyzed their impact on HCV infection
8 by combining computational and functional approaches.

9

10 **Material and methods**

11 **Cells, cell culture conditions, viruses, virus purification, infectivity assays, miRNAs,**
12 **antagomiRs, siRNAs, antibodies, immunoblot, immunocapture, electron microscopy**
13 **analysis of viral particles and gene expression analysis in liver tissue** are described in
14 the Supplementary information.

15

16 **Functional miRNA/siRNA screens.** Huh7.5.1 cells were transfected with the miRIDIAN
17 human miRNA mimic library (miRBase 19) comprising more than 2000 mature miRNAs or 28
18 ON-TARGETplus smart pool siRNAs (20 nM, Dharmacon) using Interferin HTS (Polyplus) in
19 a 96-well format[6]. After 48h, a viability test (Presto Blue, Thermo Scientific) was performed
20 prior to a two-step infection assay[15, 16, 17]. During part 1 of the protocol, 50 μ L of HCV cell
21 culture-derived particles (HCVcc, JcR2a) were incubated with cells during 4h. The inoculum
22 was removed and cells were incubated with 150 μ l of medium for 48h. In part 2, supernatants
23 from part 1 cells were transferred onto naïve Huh7.5.1 cells and part 1 cells were lysed to
24 determine luciferase activity[17, 18]. After 72h, part 2 cells were lysed to determine luciferase
25 activity[17]. siCD81 (20 nM), antagomiR-122 (100 nM) and siApoE (20 nM) were used as
26 positive controls[17]. A non-targeting siRNA with no sequence complementarity to any
27 human gene or homology to any human miRNA was used as negative control.

28

1 **Inhibitor treatment.** Four hours following HCV RNA electroporation[6], Huh7.5.1 cells were
2 incubated with vehicle or inhibitors of OGT (peracetylated 5-thio-N-acetylglucosamine
3 (Ac₄5S-GlcNAc)[19]) or OGA (Thiamet G (Sigma))[20]. After 96h, supernatants were
4 transferred onto naïve Huh7.5.1 cells for 72h prior to determination of luciferase activity while
5 electroporated cells were lysed to determine luciferase activity.

6

7 **Gene expression analyses.** Total RNA was purified[17] and transcribed into cDNA using
8 Maxima reverse transcriptase (Thermo Scientific). *GAPDH* and *OGT* mRNA was detected by
9 real time qPCR using iTaq™ Universal Probes Supermix (Bio-Rad) and TaqMan Gene
10 Expression Assay (Thermo Scientific). Relative *OGT/GAPDH* gene expression was
11 calculated by the $\Delta\Delta C_t$ method[21].

12

13 **Dual luciferase reporter gene assay.** The human *OGT* 3'UTR sequence was retrieved from
14 NCBI (NM_181672.2) and Ensembl genome browser (ENST00000373719.3). A fragment of
15 the *OGT* 3'UTR (positions 3380-3837, NM_181672.2) (Thermo Fisher Scientific GENEART)
16 was cloned between the *NotI* and *XhoI* sites downstream of a *Renilla* luciferase cassette in a
17 psiCHECK2 plasmid (Promega). A mutated version of this construct (9-bp substitution in the
18 predicted miR-501-3p target site) was generated as described[22]. The functionality of the
19 *OGT* 3'UTR was assessed as described[23]. The miRIDIAN mimic negative control 1 was
20 used as control. *Renilla* and *firefly* luciferase activity was assessed 48h after transfection into
21 HeLa cells using Dual-Luciferase Reporter assay (Promega).

22

23 **Bioinformatic and statistical analysis.** Data analysis and statistical treatment for the
24 miRNA mimic screen were performed in R (www.r-project.org). Cell measurement data used
25 in further analysis were cell viability and luciferase activity. In total 26 sets of plates
26 (performed in triplicate) were tested. The presence of multiple wells with negative and
27 positive controls on each plate allowed stepwise normalization intra- and inter-plate. First,
28 intra-plate zonal bias was examined and a model of median effects across the entire screen

1 determined using the median-polish algorithm[24] and all plates corrected accordingly. Then
2 the dataset was examined for outlier plates, i.e. plates where all individual measurements
3 correlate very poorly with the other remaining replicates. Three and 9 plates were excluded
4 for part 1 and part 2 of the screen, respectively, based on poor median correlation ($r < 0.7$)
5 so that the remaining plates correlation improved substantially ($> 40\%$). Next, the plates were
6 normalized inter replicates using the particularly robust quantile-quantile approach[25].
7 Finally, the data were tested using a moderated t-test (empirical Bayes shrinkage, R-
8 package limma[26]) for the null-hypothesis of no change of a given miRNA compared to the
9 negative control. The resulting p -values for independent testing of each miRNA were
10 corrected for the multiple testing situation and expressed as local false discovery rate (lfd,
11 R-package fdrtool[27]). The testing was performed independently for part 1 and 2 of the
12 screen and candidate miRNAs selected for each part. For data from part 1, a lfd threshold of
13 0.00027 was used. Data from part 2 were subject to increase inherent stochastic noise and
14 for this reason the minimum acceptable relative risk of false positives was increased to
15 0.1226 (i.e. maximum 15% risk for each of the retained hits).

16 Other datasets were analyzed using the two-tailed Mann-Whitney test, Wilcoxon test,
17 Spearman correlation or the two-tailed unpaired t-test for data with normal distribution as
18 assessed by D'Agostino and Pearson omnibus and Shapiro-Wilk normality tests (GraphPad
19 Prism v.6 package).

20

21

22 **Results**

23 **Genome-wide identification of human miRNAs affecting the HCV life cycle.** We
24 performed a genome-wide screen in human hepatoma Huh7.5.1 cells using a genomic
25 miRNA mimics library and a two-step infection assay[17] with a luciferase reporter virus
26 (JcR2a), which allowed us to functionally assess the role of miRNAs during the early steps
27 (part 1 - viral entry/translation/replication) and the late steps (part 2 - viral
28 assembly/release/infectivity) of the HCV life cycle (Fig. 1A). Silencing of *CD81* and *ApoE*,

1 two essential host factors required for HCV entry or assembly, respectively, was performed
2 in parallel using small interfering RNA (siRNA) as controls. Silencing of *CD81* resulted in a
3 reduction of HCV infection in part 1 and consequently in part 2 of the screen since reduced
4 viral entry in the first part of the assay leads to a reduced production of viral particles (Fig.
5 1B)[17]. Silencing of *ApoE* resulted in a marked inhibition of HCV infection only in part 2 of
6 the assay, consistent with the role of ApoE in HCV assembly (Fig. 1B)[17]. The screen
7 identified 427 miRNAs (corresponding to about 16% of the library) that significantly
8 modulated HCV infection (l_{fdr} < threshold, Supplementary Table 1 and Fig. 1C): 186 miRNAs
9 affected HCV infection in part 1, 309 miRNAs affected HCV infection in part 2, including 68
10 hits in part 1 and part 2. The limited number of part 1 and 2 hits may be due to the fact that a
11 single miRNA may modulate the expression of several proteins, which may have different
12 roles in the viral life cycle. Most hits were observed to dampen HCV infection independently
13 of any significant alteration of cell viability (data not shown). The 186 miRNAs modulating the
14 early steps of HCV infection all decreased viral infection. Among the 309 miRNAs that had
15 an impact in part 2, 11 miRNAs increased HCV infection by at least 3-fold while 298 miRNAs
16 inhibited HCV infection by at least 2.7-fold. Hits from the screen included the let-7 family[12,
17 28], miR-27a[29] and miR-29 family[30] that were already shown to inhibit HCV infection, as
18 well as miR-21[31] and miR-146a-5p[17] that were shown to stimulate HCV infection thus
19 supporting the relevance of our findings. Collectively, our screen identified a set of miRNAs
20 whose overexpression overall impairs HCV infection by affecting viral
21 entry/translation/replication and/or virion assembly/egress/infectivity.

22

23 **miR-619-3p, miR-501-3p and OGT play a role in late steps of the HCV life cycle.** We
24 focused our analysis on miRNAs that modulate late steps of the HCV life cycle, as the
25 molecular mechanisms of HCV assembly/release remain only partially understood. Our
26 screen identified 241 miRNAs that modulated late steps without affecting early steps of
27 infection: 11 miRNAs increased HCV infection while 230 miRNAs decreased HCV infection.
28 Among the miRNAs that increased HCV infection, miR-140-3p, miR-501-3p, miR-619-3p and

1 miR-4778-5p have not yet been associated with HCV. Since they enhanced HCV infection in
2 part 2 without affecting part 1, these miRNAs may target host genes that control virus
3 assembly/egress/infectivity. We first confirmed the effect of these miRNAs in independent
4 experiments using the same protocol as for the screen. Overexpression of miR-619-3p or
5 miR-501-3p consistently led to an increase in the infection of progeny virions (Fig. 1D) while
6 infection was decreased with progeny virions from antagomiR-transfected cells
7 (Supplementary Figure S1A). miR-619-3p or miR-501-3p were thus selected for further
8 investigation. To study the molecular mechanisms by which these miRNAs affect HCV
9 infection, we generated a list of predicted miRNA targets using DIANA, TargetScan Human
10 v6.2 and miRDB databases, and selected candidate targets based on their expression in our
11 Huh7.5.1 cells as assessed by microarray (data not shown). Ingenuity Pathway Analysis
12 enabled us to refine the gene list by selecting 28 genes involved in the following functional
13 networks or pathways that contribute to the HCV life cycle[32, 33, 34]: lipid metabolism and
14 cholesterol biosynthesis, protein maturation and processing at the endoplasmic reticulum
15 (ER), components of the endosomal sorting complex, adipocyte biogenesis, cellular
16 morphology and cell inflammation (Table 1).

17 To assess whether knock-down of these 28 candidate targets affects virus
18 production, we performed a siRNA-based screen using siRNA pools exhibiting strong
19 silencing without cytotoxicity (Fig. 2). Silencing of *CD81* and antagomiR-122 served as
20 controls for part 1; knock-down of *ApoE* served as control for part 2 (Fig. 2). Hits were
21 defined as genes whose knock-down modulated HCV infection in at least one part of the
22 screen with high significance (Fig. 2, p -value < 0.0001, Mann-Whitney U-test). HCV
23 entry/translation/replication was significantly modulated by silencing of *PPP3CA*, *CEBPA*,
24 *MID1*, *WDFY3*, *DCX* and *SLC35D1*. HCV assembly/egress/infectivity was significantly
25 modulated by knock-down of *PPP3CA*, *CSDE1*, *GAN*, *USP37*, *CEBPA*, *MID1*, *WDFY3*, *DCX*,
26 *MAPK9*, *SLC35D1*, *DCC*, *RNF144A*, *PPP2R2C* and *OGT*. Strikingly, only the silencing of
27 *OGT* was associated with an enhancement of HCV assembly/release/infectivity (p -value =
28 0.0002), while that of the other hits was associated with reduced HCV infection (Fig. 2).

1 These results indicate that the down-regulation of *OGT* phenocopies the effect of miR-501-
2 3p and miR-619-3p on HCV infection (Fig. 2) and suggest *OGT* as a novel player in the HCV
3 life cycle.

4
5 **miR-501-3p post-transcriptionally regulates *OGT* expression.** To study whether miR-501-
6 3p and miR-619-3p target *OGT*, we analyzed *OGT* RNA and protein levels in Huh7.5.1 cells
7 following overexpression of miR-501-3p or miR-619-3p. While neither miRNA had an impact
8 on *OGT* RNA levels (Fig. 3A), up-regulation of miR-501-3p significantly decreased *OGT*
9 protein expression by ~65% (Fig. 3B, p -value < 0.05, t-test). miR-619-3p also decreased
10 *OGT* expression but less robustly than miR-501-3p (Fig. 3B), prompting us to focus our
11 investigation on miR-501-3p. To assess whether *OGT* is a functional target of miR-501-3p,
12 we subcloned a fragment of the *OGT* mRNA 3'UTR that harbors the predicted miR-501-3p
13 target site in the *Renilla* luciferase expression cassette (RLuc) of a dual luciferase reporter
14 construct. Co-transfection of miR-501-3p mimic with the wild-type 3'UTR reporter (RLuc wt
15 *OGT* 3'UTR) significantly decreased luciferase activity as compared to the empty vector (Fig.
16 3C, p -value < 0.05, t-test). In contrast, the repression of luciferase expression was lost when
17 the reporter with mutated miR-501-3p binding site (RLuc mt *OGT* 3'UTR) was used (Fig. 3C).
18 These data are consistent in indicating that miR-501-3p mediates post-transcriptional
19 regulation of *OGT*.

20
21 **O-GlcNAcylation modulates HCVcc infectivity.** To investigate whether *OGT* modulates
22 HCV assembly and/or infectivity, we determined infectious virus titer (TCID₅₀) and HCV RNA
23 levels to calculate the specific infectivity of HCVcc particles generated in *OGT*-silenced
24 Huh7.5.1 cells. Interestingly, *OGT*-silencing led to a significant increase in the TCID₅₀ and
25 the specific infectivity of HCVcc (Fig. 4A, p -value < 0.05, Mann-Whitney test). Noteworthy,
26 the effect of *OGT* on HCVcc infectivity was genotype-independent as demonstrated by
27 increased infectivity of HCVcc bearing the envelope glycoproteins of genotypes 1a, 1b and
28 2a upon *OGT*-silencing (Fig. 4B). We next sought to investigate how *OGT* could modulate

1 HCVcc infectivity. OGT is the only enzyme that catalyzes the addition of N-
2 acetylglucosamine (O-GlcNAc) to serine and threonine residues of proteins. Moreover, OGT
3 has a scaffold function and promotes binding of proteins in multiprotein complexes[35]. To
4 assess whether the enzymatic activity of OGT modulates HCVcc infectivity, we used
5 pharmacological inhibitors of OGT (Ac₄5S-GlcNAc) or O-GlcNAcase (OGA) (Thiamet G), the
6 OGT counterpart that removes O-GlcNAc (Fig. 4C). Ac₄5S-GlcNAc led to a significant
7 enhancement of HCVcc infectivity in a dose-dependent manner, while the opposite effect
8 was observed with Thiamet G (Fig. 4D, *p*-value < 0.05, Mann-Whitney test). Collectively,
9 these results demonstrate that O-GlcNAcylation modulates HCVcc infectivity.

10

11 **OGT-silencing affects HCVcc biophysical properties and size distribution.** To further
12 assess how OGT may impact HCVcc morphogenesis, we analyzed the structural and
13 biophysical properties of HCVcc produced in siCtrl- and siOGT-transfected Huh7.5.1 cells
14 following iodixanol gradient ultracentrifugation. Silencing of OGT led to the production of
15 more infectious HCVcc with higher density (Fig. 5A-B) as well as higher ApoE concentrations
16 (Fig. 5C) suggesting that OGT/O-GlcNAcylation affects the biophysical properties of HCVcc.
17 No change in apoB concentrations were observed between HCVcc produced from siCtrl- or
18 siOGT-transfected cells (Fig. 5D), in line with the model that HCV lipovirions contain
19 several exchangeable ApoE molecules and one non-exchangeable apoB[36]. We also
20 visualized HCVcc by electron microscopy (EM) following anti-E2 antibody
21 immunocapture[36] to assess whether OGT-silencing had an impact on HCVcc size. Particle
22 size distribution was assessed from a series of randomly acquired electron micrographs. A
23 shift towards bigger sizes was observed for sucrose-cushion purified HCVcc generated in
24 OGT-silenced Huh7.5.1 cells as compared to control HCVcc (Fig. 6A-B). This shift was also
25 observed in different fractions of iodixanol gradient-separated HCVcc (Fig. 6C-F) in line with
26 the higher infectivity and ApoE concentrations of HCVcc generated in OGT-silenced
27 Huh7.5.1 cells (Fig. 5A-C). These data suggest that OGT-silencing affects the lipidation of
28 HCVcc.

1 **OGT expression increases in liver disease.** Since silencing of OGT promotes HCV
2 infectivity, we assessed whether HCV infection in turn had an effect on miR-501-3p and OGT
3 expression. In Huh7.5.1 cells, HCV infection lead to small but significant increase of miR-
4 501-3p and decrease of OGT levels (Fig. 7A-B and Supplementary Fig. 1B; p -value < 0.05,
5 Mann-Whitney test), which may promote viral infection given the pro- and antiviral roles of
6 miR-501-3p and O-GlcNAcylation, respectively (Fig. 1C-D and 4D). In contrast, no significant
7 difference of OGT expression was observed between the livers of HCV transgenic and wild-
8 type mice[37] (data not shown) suggesting that HCV proteins do not directly modulate OGT
9 expression. In liver tissue from HCV-infected patients, HCV RNA levels were not correlated
10 with OGT expression (Fig. 7C, Spearman correlation: 0.06004019, p -value = 0.7661)
11 suggesting that in patients there is likely no direct effect of HCV on OGT expression.

12 O-GlcNAcylation has been associated with a variety of cancers, including HCC
13 recurrence linked to increased O-GlcNAcylation after liver transplantation[38]. We therefore
14 investigated OGT expression in chronic liver disease and HCC. While there was a trend for
15 increased OGT expression in liver tissue from HCV-infected patients with fibrosis and
16 inflammation (Fig. 7D-E), OGT levels were markedly and significantly elevated in the tumor
17 liver tissue of patients chronically infected with HCV or hepatitis B virus and patients with
18 alcoholic liver disease or non-alcoholic fatty liver disease as compared to non-tumor tissue
19 (Fig. 7F, p -value < 0.05, Wilcoxon test). These data suggest that OGT expression increases
20 in HCC in an etiology-independent manner. Collectively, these results suggest that OGT
21 expression is likely increased in HCV-induced liver disease and cancer through inflammation
22 and fibrosis rather than by HCV itself.

23

24

25 **Discussion**

26 By focusing on miRNAs affecting late steps of the viral life cycle, we uncovered that i) miR-
27 501-3p regulates the expression of OGT; ii) silencing of OGT expression or inhibition of its
28 enzymatic activity increases the infectivity of HCV particles; and iii) OGT knock-down leads

1 to the release of bigger HCV particles. Our data suggest that O-GlcNAcylation affects HCV
2 morphogenesis and infectivity.

3 While we were characterizing the role of OGT/O-GlcNAcylation for HCV
4 morphogenesis, Li and colleagues published their functional genomics study of HCV-miRNA
5 interactions[12]. By conducting genome wide miRNA mimic and hairpin inhibitor screens,
6 they identified a set of miRNAs exhibiting a pro- or antiviral effect on HCV. Characterization
7 of the underlying molecular processes showed that miR-25, let-7 and miR-130 families
8 restrict viral infection by decreasing the expression of cellular HCV co-factors[12]. Despite
9 similarities in the cell type and HCV infection models used here and by Li and colleagues,
10 our screen only displays a small overlap with their study (9% common miRNA hits). This is
11 not surprising given the small overlap between previous siRNA screens to uncover HCV host
12 factors[6, 15] and is likely due i) to the different sizes of miRNA mimic libraries as the library
13 used here was more than 2-times larger than the one used by Li and co-workers, and ii) to
14 the markedly distinct pipelines for hit selection that were used in the two studies.
15 Nonetheless, both screens were consistent in confirming the proviral role of miR-146a-5p in
16 promoting HCV assembly/egress that we previously reported[17] and the global multistep
17 inhibitory effects of the let-7 family on HCV infection[28], further corroborating the
18 involvement of these miRNAs in fine-tuning the HCV life cycle. Both studies also consistently
19 indicated that miR-518a-5p, miR-517-3p, miR-185 and members of the miR-302 family inhibit
20 early steps of HCV infection, while miR-586, miR-620 and members of the miR-200 family
21 inhibit late steps of viral infection. Since none of these miRNAs except miR-185 has been
22 previously associated with HCV infection[39], it might be interesting to further characterize
23 the involvement of these miRNAs in HCV-host interactions. Interestingly, an overall proviral
24 effect of miR-501-3p was also observed by Li and colleagues[12], however the mechanism of
25 action was not studied. By characterizing the role of miR-501-3p in the HCV life cycle, we
26 uncovered OGT as a miR-501-3p target in liver-derived cells and showed for the first time a
27 link between O-GlcNAcylation and HCV infection. These results indicate that genome-wide

1 miRNA functional screens represent a powerful strategy to dissect the role of miRNAs in
2 pathogen-host interactions.

3 While N-glycosylation of HCV envelope glycoproteins plays an important role for
4 escape from virus-neutralizing antibodies[40], so far no functional association between HCV
5 and O-glycosylation has been reported. In contrast to N-linked glycosylation that consists of
6 the attachment of a glycan to a nitrogen of an asparagine residue of proteins in the ER/Golgi
7 prior to their trafficking to the plasma membrane and/or their secretion, the glycosylation of
8 serine and threonine residues with O-GlcNAc is a post-translational modification (PTM) of
9 intracellular proteins that are localized in the nucleus, cytoplasm or mitochondria. The O-
10 glycosylation/deglycosylation of proteins is catalyzed by a single pair of nucleo-cytoplasmic
11 enzymes, OGT/OGA. O-GlcNAcylation is complementary to protein
12 phosphorylation/dephosphorylation, another more broadly known abundant protein PTM that
13 involves numerous kinases/phosphatases. OGT/OGA are often found in protein complexes
14 that also include kinases/phosphatases and a protein can be either O-GlcNAcylated or
15 phosphorylated on a same residue to fine-tune cellular signaling[41]. O-GlcNAcylation and
16 phosphorylation on the same or neighboring serine or threonine residue is known as yin yang
17 site[42].

18 O-GlcNAcylation plays a major role in the regulation of metabolic pathways in the
19 liver, including insulin signaling, bile acid metabolism and lipogenesis[35]. The large number
20 of OGT/OGA substrates and cellular pathways regulated by O-GlcNAcylation hampers a
21 detailed characterization of the role of these proteins in HCV infection. Since i) HCV
22 assembly takes place at ER-derived membranes, ii) OGT/OGA are not known to localize in
23 the ER lumen, and iii) O-GlcNAcylation of extracellular proteins containing EGF-like domains
24 is catalyzed by EGF domain-specific OGT (EOGT) in the ER lumen in an OGT-independent
25 manner[43]), OGT/OGA most likely modulate HCV infection by post-translationally modifying
26 one or several cellular factors required for HCV morphogenesis rather than by affecting viral
27 proteins, although HCV glycoproteins contain putative O-GlcNAcylation sites as determined
28 using OGlcNAcScan, OGTsite and YingOYang1.2 bioinformatics tools (data not shown).

1 Regarding HCV host factors that may be regulated by OGT/OGA, O-GlcNAcylation
2 sites have been predicted in human CLDN1[44] and OCLN at serine sites that can also be
3 phosphorylated and this has been suggested to potentially play a role for HCV entry[45].
4 However, in our experimental setting we did not observe a significant effect of OGT-silencing
5 on the early steps of HCV infection, suggesting that O-GlcNAcylation of CLDN1 and/or
6 OCLN likely does not play a major role in HCV infection. Other host factors important for the
7 HCV life cycle are well-known O-GlcNAcylated proteins, as for example various nuclear pore
8 complex proteins (Nups) including Nup98, Nup153 and Nup155 that are involved in HCV
9 replication and assembly and/or may be associated with viral particles[46, 47, 48]. However,
10 since depletion of Nups was reported to alter HCV replication and/or assembly but to have
11 no impact on the specific infectivity of HCV particles[46] in contrast to the depletion of OGT
12 as shown here, it is unlikely that a modulation of Nup O-GlcNAcylation accounts for the
13 effects of OGT-silencing and/or OGT/OGA inhibitors on HCVcc infectivity observed in our
14 study. This is in line with our observation that OGT knock-down had no effect on Dengue
15 virus (DENV) replication and infectivity (data not shown), although Nup98 had been
16 suggested to potentially play a role for DENV infection[46]. These data suggest that OGT
17 does not broadly modulate the infectivity of viruses of the *Flaviviridae* family.

18 However, OGT and/or O-GlcNAcylation have been reported to play a role in the
19 infection with other viruses[49, 50, 51]. Interestingly, while OGT expression modulates the
20 levels of human papillomavirus 16 (HPV16) oncoproteins E6 and E7[52], E6 in turn can up-
21 regulate OGT to increase O-GlcNAcylation and the oncogene activities of HPV[53],
22 suggesting that OGT/O-GlcNAcylation could play a role in virus-induced cancer. In cell
23 culture, HCV infection appeared to be associated with a minor decrease in OGT expression
24 in line with an antiviral role of O-GlcNAcylation. In contrast, an increased OGT expression
25 was observed in HCC tissues of HCV-infected patients. Since OGT has been suggested to
26 activate oncogenic signaling pathways in non-alcoholic steatohepatitis-related HCC[54] and
27 O-GlcNAcylation has been associated with HCC recurrence linked to increased O-
28 GlcNAcylation after liver transplantation[38], these data suggest that in addition to their effect

1 on the HCV life cycle, OGT/O-GlcNAcylation may also play a role in HCV-induced
2 hepatocarcinogenesis.

3

4

5 **Acknowledgments**

6 We wish to thank Gerald W. Hart and Stéphan Hardivillé, the CardioPEG CoreC4 (NHLBI
7 P01 HL107153) for providing AL24 and for useful technical discussions. We are grateful to
8 David Vocadlo (Simon Fraser University, Burnaby, Canada) for the gift of Ac₄S-GlcNAc. We
9 also thank Ralf Bartenschlager (University of Heidelberg, Germany) for providing the
10 plasmids for production of HCVcc and Frank Chisari (The Scripps Research Institute, La
11 Jolla, CA) for the gift of Huh7.5.1 cells. We acknowledge Evelyne Schaeffer (CNRS
12 UPR3572, IBMC, Strasbourg) for the DENV experiment, Charlotte Bach and Christine
13 Thumann (Inserm, U1110, Strasbourg) for excellent technical work during the functional
14 miRNA mimic screen, as well as Armando A. Roca-Suarez (Inserm, U1110, Strasbourg),
15 Hussein El Saghire (Inserm, U1110, Strasbourg), Arnaud Kopp (IGBMC, Department of
16 Functional Genomics and Cancer) and Erika Girardi (UPR 9002, IBMC, Strasbourg) for
17 helpful discussions. We thank the INGESTEM infrastructure for access to the IGBMC high-
18 throughput screening workstation.

19

20

21 **References**

22 1 Baumert TF, Juhling F, Ono A, *et al.* Hepatitis C-related hepatocellular carcinoma in
23 the era of new generation antivirals. *BMC Med* 2017;**15**:52.

24 2 Pawlotsky JM. Hepatitis C Virus Resistance to Direct-Acting Antiviral Drugs in
25 Interferon-Free Regimens. *Gastroenterology* 2016;**151**:70-86.

26 3 Dietz J, Susser S, Vermehren J, *et al.* Patterns of Resistance-Associated
27 Substitutions in Patients With Chronic HCV Infection Following Treatment With Direct-Acting
28 Antivirals. *Gastroenterology* 2018;**154**:976-88 e4.

1 4 Zeisel MB, Baumert TF. Clinical development of hepatitis C virus host-targeting
2 agents. *Lancet* 2017;**389**:674-5.

3 5 Zeisel MB, Crouchet E, Baumert TF, *et al.* Host-Targeting Agents to Prevent and
4 Cure Hepatitis C Virus Infection. *Viruses* 2015;**7**:5659-85.

5 6 Lupberger J, Zeisel MB, Xiao F, *et al.* EGFR and EphA2 are host factors for hepatitis
6 C virus entry and possible targets for antiviral therapy. *Nature Medicine* 2011;**17**:589-95.

7 7 Fuchs BC, Hoshida Y, Fujii T, *et al.* Epidermal growth factor receptor inhibition
8 attenuates liver fibrosis and development of hepatocellular carcinoma. *Hepatology*
9 2014;**59**:1577-90.

10 8 Jopling CL, Yi M, Lancaster AM, *et al.* Modulation of hepatitis C virus RNA
11 abundance by a liver-specific MicroRNA. *Science* 2005;**309**:1577-81.

12 9 Masaki T, Arend KC, Li Y, *et al.* miR-122 stimulates hepatitis C virus RNA synthesis
13 by altering the balance of viral RNAs engaged in replication versus translation. *Cell Host*
14 *Microbe* 2015;**17**:217-28.

15 10 Janssen HL, Reesink HW, Lawitz EJ, *et al.* Treatment of HCV infection by targeting
16 microRNA. *N Engl J Med* 2013;**368**:1685-94.

17 11 van der Ree MH, de Vree JM, Stelma F, *et al.* Safety, tolerability, and antiviral effect
18 of RG-101 in patients with chronic hepatitis C: a phase 1B, double-blind, randomised
19 controlled trial. *Lancet* 2017;**389**:709-17.

20 12 Li Q, Lowey B, Sodroski C, *et al.* Cellular microRNA networks regulate host
21 dependency of hepatitis C virus infection. *Nat Commun* 2017;**8**:1789.

22 13 Bandiera S, Pfeffer S, Baumert TF, *et al.* miR-122--a key factor and therapeutic target
23 in liver disease. *J Hepatol* 2015;**62**:448-57.

24 14 Li H, Jiang JD, Peng ZG. MicroRNA-mediated interactions between host and hepatitis
25 C virus. *World J Gastroenterol* 2016;**22**:1487-96.

26 15 Li Q, Brass AL, Ng A, *et al.* A genome-wide genetic screen for host factors required
27 for hepatitis C virus propagation. *Proc Natl Acad Sci U S A* 2009;**106**:16410-5.

1 16 Poenisch M, Metz P, Blankenburg H, *et al.* Identification of HNRNPK as regulator of
2 hepatitis C virus particle production. *PLoS Pathog* 2015;**11**:e1004573.

3 17 Bandiera S, Pernot S, El Saghire H, *et al.* Hepatitis C Virus-Induced Upregulation of
4 MicroRNA miR-146a-5p in Hepatocytes Promotes Viral Infection and Deregulates Metabolic
5 Pathways Associated with Liver Disease Pathogenesis. *J Virol* 2016;**90**:6387-400.

6 18 Da Costa D, Turek M, Felmlee DJ, *et al.* Reconstitution of the entire hepatitis C virus
7 life cycle in non-hepatic cells. *J Virol* 2012;**86**:11919-25.

8 19 Gloster TM, Zandberg WF, Heinonen JE, *et al.* Hijacking a biosynthetic pathway
9 yields a glycosyltransferase inhibitor within cells. *Nat Chem Biol* 2011;**7**:174-81.

10 20 Yuzwa SA, Macauley MS, Heinonen JE, *et al.* A potent mechanism-inspired O-
11 GlcNAcase inhibitor that blocks phosphorylation of tau in vivo. *Nat Chem Biol* 2008;**4**:483-90.

12 21 Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T)
13 method. *Nat Protoc* 2008;**3**:1101-8.

14 22 Jin Y, Chen Z, Liu X, *et al.* Evaluating the microRNA targeting sites by luciferase
15 reporter gene assay. *Methods Mol Biol* 2013;**936**:117-27.

16 23 Van Renne N, Roca Suarez AA, Duong FHT, *et al.* miR-135a-5p-mediated
17 downregulation of protein tyrosine phosphatase receptor delta is a candidate driver of HCV-
18 associated hepatocarcinogenesis. *Gut* 2018;**67**:953-62.

19 24 Mosteller F, Tukey J. *Data Analysis and Regression*. Reading, MA: Addison-Wesley,
20 1977.

21 25 Amaratunga D, Cabrera J. Analysis of Data from Viral DNA Microchips. *Journal of the*
22 *American Statistical Association* 2001;**96**:1161.

23 26 Smyth GK. Linear models and empirical Bayes methods for assessing differential
24 expression in microarray experiments. *Statistical Applications in Genetics and Molecular*
25 *Biology* 2004;**3**:Article 3.

26 27 Strimmer K. *fdRtool*: a versatile R package for estimating local and tail area-based
27 false discovery rates. *Bioinformatics* 2008;**24**:1461-2.

1 28 Cheng M, Si Y, Niu Y, *et al.* High-throughput profiling of alpha interferon- and
2 interleukin-28B-regulated microRNAs and identification of let-7s with anti-hepatitis C virus
3 activity by targeting IGF2BP1. *J Virol* 2013;**87**:9707-18.

4 29 Shirasaki T, Honda M, Shimakami T, *et al.* MicroRNA-27a regulates lipid metabolism
5 and inhibits hepatitis C virus replication in human hepatoma cells. *J Virol* 2013;**87**:5270-86.

6 30 Bandyopadhyay S, Friedman RC, Marquez RT, *et al.* Hepatitis C virus infection and
7 hepatic stellate cell activation downregulate miR-29: miR-29 overexpression reduces
8 hepatitis C viral abundance in culture. *J Infect Dis* 2011;**203**:1753-62.

9 31 Chen Y, Chen J, Wang H, *et al.* HCV-induced miR-21 contributes to evasion of host
10 immune system by targeting MyD88 and IRAK1. *PLoS Pathog* 2013;**9**:e1003248.

11 32 Ariumi Y, Kuroki M, Maki M, *et al.* The ESCRT system is required for hepatitis C virus
12 production. *PLoS One* 2011;**6**:e14517.

13 33 Paul D, Madan V, Bartenschlager R. Hepatitis C virus RNA replication and assembly:
14 living on the fat of the land. *Cell Host Microbe* 2014;**16**:569-79.

15 34 Meyers NL, Fontaine KA, Kumar GR, *et al.* Entangled in a membranous web: ER and
16 lipid droplet reorganization during hepatitis C virus infection. *Curr Opin Cell Biol* 2016;**41**:117-
17 24.

18 35 Yang X, Qian K. Protein O-GlcNAcylation: emerging mechanisms and functions. *Nat*
19 *Rev Mol Cell Biol* 2017;**18**:452-65.

20 36 Piver E, Boyer A, Gaillard J, *et al.* Ultrastructural organisation of HCV from the
21 bloodstream of infected patients revealed by electron microscopy after specific
22 immunocapture. *Gut* 2017;**66**:1487-95.

23 37 Lerat H, Honda M, Beard MR, *et al.* Steatosis and liver cancer in transgenic mice
24 expressing the structural and nonstructural proteins of hepatitis C virus. *Gastroenterology*
25 2002;**122**:352-65.

26 38 de Queiroz RM, Carvalho E, Dias WB. O-GlcNAcylation: The Sweet Side of the
27 Cancer. *Front Oncol* 2014;**4**:132.

1 39 Singaravelu R, O'Hara S, Jones DM, *et al.* MicroRNAs regulate the immunometabolic
2 response to viral infection in the liver. *Nat Chem Biol* 2015;**11**:988-93.

3 40 Lavie M, Hanouille X, Dubuisson J. Glycan Shielding and Modulation of Hepatitis C
4 Virus Neutralizing Antibodies. *Front Immunol* 2018;**9**:910.

5 41 Hart GW, Slawson C, Ramirez-Correa G, *et al.* Cross talk between O-GlcNAcylation
6 and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu Rev*
7 *Biochem* 2011;**80**:825-58.

8 42 Hart GW, Greis KD, Dong LY, *et al.* O-linked N-acetylglucosamine: the "yin-yang" of
9 Ser/Thr phosphorylation? Nuclear and cytoplasmic glycosylation. *Adv Exp Med Biol*
10 1995;**376**:115-23.

11 43 Sakaidani Y, Nomura T, Matsuura A, *et al.* O-linked-N-acetylglucosamine on
12 extracellular protein domains mediates epithelial cell-matrix interactions. *Nat Commun*
13 2011;**2**:583.

14 44 Butt AM, Khan IB, Hussain M, *et al.* Role of post translational modifications and novel
15 crosstalk between phosphorylation and O-beta-GlcNAc modifications in human claudin-1, -3
16 and -4. *Mol Biol Rep* 2012;**39**:1359-69.

17 45 Butt AM, Feng D, Nasrullah I, *et al.* Computational identification of interplay between
18 phosphorylation and O-beta-glycosylation of human occludin as potential mechanism to
19 impair hepatitis C virus entry. *Infect Genet Evol* 2012;**12**:1235-45.

20 46 Neufeldt CJ, Joyce MA, Levin A, *et al.* Hepatitis C virus-induced cytoplasmic
21 organelles use the nuclear transport machinery to establish an environment conducive to
22 virus replication. *PLoS Pathog* 2013;**9**:e1003744.

23 47 Lussignol M, Kopp M, Molloy K, *et al.* Proteomics of HCV virions reveals an essential
24 role for the nucleoporin Nup98 in virus morphogenesis. *Proc Natl Acad Sci U S A*
25 2016;**113**:2484-9.

26 48 Zhu Y, Liu TW, Madden Z, *et al.* Post-translational O-GlcNAcylation is essential for
27 nuclear pore integrity and maintenance of the pore selectivity filter. *J Mol Cell Biol* 2016;**8**:2-
28 16.

1 49 Jochmann R, Thureau M, Jung S, *et al.* O-linked N-acetylglucosaminylation of Sp1
2 inhibits the human immunodeficiency virus type 1 promoter. *J Virol* 2009;**83**:3704-18.

3 50 Groussaud D, Khair M, Tollenaere AI, *et al.* Hijacking of the O-GlcNAcZYME complex
4 by the HTLV-1 Tax oncoprotein facilitates viral transcription. *PLoS Pathog*
5 2017;**13**:e1006518.

6 51 Angelova M, Ortiz-Meoz RF, Walker S, *et al.* Inhibition of O-Linked N-
7 Acetylglucosamine Transferase Reduces Replication of Herpes Simplex Virus and Human
8 Cytomegalovirus. *J Virol* 2015;**89**:8474-83.

9 52 Kim M, Kim YS, Kim H, *et al.* O-linked N-acetylglucosamine transferase promotes
10 cervical cancer tumorigenesis through human papillomaviruses E6 and E7 oncogenes.
11 *Oncotarget* 2016;**7**:44596-607.

12 53 Zeng Q, Zhao RX, Chen J, *et al.* O-linked GlcNAcylation elevated by HPV E6
13 mediates viral oncogenesis. *Proc Natl Acad Sci U S A* 2016;**113**:9333-8.

14 54 Xu W, Zhang X, Wu JL, *et al.* O-GlcNAc transferase promotes fatty liver-associated
15 liver cancer through inducing palmitic acid and activating endoplasmic reticulum stress. *J*
16 *Hepatol* 2017;**67**:310-20.

17

1 **Figure legends**

2 **Figure 1. High-throughput screen identifies human miRNAs that regulate the HCV life**
3 **cycle.** (A) Schematic outline of the miRNA mimic screen strategy. Huh7.5.1 cells were
4 transfected with miRNA mimics or controls prior to infection with *Renilla* luciferase HCVcc
5 (JcR2a) two days later (part 1). Cell supernatants of part 1 were used to inoculate naïve
6 Huh7.5.1 cells (part 2). Cells from part 1 and part 2 were lysed at the end of each infection
7 step (2 and 3 days post infection, respectively) to determine luciferase activity. (B)
8 Modulation of HCV entry and replication (part 1) and/or assembly and infectivity (part 2) upon
9 transfection of control non-targeting siRNA (siCtrl, negative control), siCD81 (inhibiting viral
10 entry) or siApoE (inhibiting viral assembly). By inhibiting HCV entry, siCD81 impacts part 1
11 as well as part 2. In contrast, by specifically impairing late steps of HCV replication cycle,
12 siApoE inhibits HCV infection only in part 2. The box plots show the sample lower quartile
13 (25th percentile; bottom of the box), the median (50th percentile; horizontal line in box) and
14 the upper quartile (75th percentile; top of the box) of relative light units (RLU) in each lysate.
15 The whiskers indicate s.d. Data are from three independent experiments. (C) Effects of
16 miRNA overexpression on each part of the HCV life cycle. Data were tested using a
17 moderated t-test (empirical Bayes shrinkage, R-package limma[26]) for the null-hypothesis of
18 no change of a given miRNA compared to the negative control. The resulting p-values for
19 independent testing of each miRNA were corrected for the multiple testing situation and
20 expressed as local false discovery rate (lfdr, R-package fdrtool[27]). miRNAs having a
21 significant effect on either part 1 or 2 of the screen are below the thresholds indicated by
22 dashed lines (lfdr < 0.00027 or 0.1226, respectively). miRNAs that were previously reported
23 to impact on HCV infection as well as miR-140-3p, miR-501-3p, miR-619-3p and miR-4778-
24 5p are highlighted in blue (Log₂(FC) < 0) or red (Log₂(FC) > 0). Data are from three
25 independent experiments. (D) Effect of miR-140-3p, miR-501-3p, miR-619-3p and miR-4778-
26 5p on the HCV life cycle. Huh7.5.1 cells were transfected with siCtrl (Ctrl), miR-140-3p, miR-
27 501-3p, miR-619-3p or miR-4778-5p and infection experiments were carried out as described
28 in A. HCV infection was determined as luciferase activity. Results represent mean

1 percentage \pm s.d. from three independent experiments in triplicate. The dashed line indicates
2 values from control-transfected cells set at 100%. Statistics: *, p -value < 0.05, Mann-Whitney
3 test.

4

5 **Figure 2. OGT is a novel host cell factor involved in the late steps of the HCV life cycle.**

6 Huh7.5.1 cells were transfected with a set of siRNAs against 28 predicted targets of miR-
7 501-3p and/or miR-619-3p, and infected with HCVcc JcR2A according to the two-step
8 protocol depicted in Fig. 1A. siCD81, antagomiR-122 and siApoE were used as loss-of-
9 function controls to perturb HCV entry, translation/replication and assembly, respectively.
10 miR-501-3p and miR-619-3p, which were ineffective in part 1 of the screen but enhanced
11 HCV infection in part 2, were transfected in parallel. HCV infection was quantified as fold
12 change of luciferase activity with respect to negative control (siCtrl). Results for different
13 replicates are shown as individual points. For each gene, median fold change of luciferase
14 activity \pm s.d. is shown as black horizontal lines. The dashed line indicates a fold change of
15 1. Data are from three independent experiments in triplicate. Results for miR-501-3p, miR-
16 619-3p and siOGT that increase HCV infection in part 2 are depicted in red. Results for
17 siRNA targeting *PPP3CA*, *CEBPA*, *MID1*, *WDFY3*, *DCX*, *SLC35D1*, *CSDE1*, *GAN*, *USP37*,
18 *MAPK9*, *DCC*, *RNF144A*, or *PPP2R2C* that significantly modulated HCV infection in part 1
19 and/or part 2 but did not phenocopy the effect of miR-501-3p and miR-619-3p are depicted in
20 blue.

21

22 **Figure 3. miR-501-3p mediates post-transcriptional regulation of OGT by decreasing**

23 **its expression at the protein level.** Huh7.5.1 cells were transfected with siCtrl (Ctrl), a pool
24 of siRNA against *OGT*, miR-501-3p or miR-619-3p. After 96h, RNA and proteins were
25 purified, and OGT expression analyzed by RT-qPCR and Western blot. (A) Percentage of
26 *OGT* mRNA expression in miRNA-transfected cells as compared to negative control. Results
27 are presented as mean \pm s.d. and are from three independent experiments in triplicate. The
28 dashed line indicates values from control-transfected cells set at 100%. Statistics: *, p -value

1 < 0.05, t-test (B) OGT protein expression. Left: percentage of OGT protein expression in
2 siRNA- or miRNA-transfected cells as assessed by quantification of Western blots. OGT
3 levels were normalized to actin levels using ImageLab™ 5.2.1 software (BioRad). Results
4 are presented as mean ± s.d. and are from three independent experiments. The dashed line
5 indicates values from control-transfected cells set at 100%. Statistics: *, *p*-value < 0.05, t-
6 test. Right: representative Western blot analysis. (C) Analysis of miRNA targeting of *OGT*
7 expression by dual luciferase reporter assay. Left: HeLa cells were co-transfected with a
8 miR-501-3p mimic and a dual luciferase reporter plasmid containing either wild type miR-
9 501-3p (RLuc wt *OGT* 3'UTR) or mutated miR-501-3p binding site (RLuc mt *OGT* 3'UTR) to
10 modulate RLuc expression. Co-transfection of the miR-501-3p mimic and empty RLuc vector
11 was used as control. Data are expressed as mean percentage of *Renilla* luciferase activity ±
12 s.d. normalized to *firefly* luciferase, and relative to co-transfection of the vectors with non-
13 targeting miRNA (miR-Ctrl). Results are from three independent experiments in triplicate.
14 The dashed line indicates values from control-transfected cells set at 100%. Statistics: *, *p*-
15 value < 0.05, t-test. Right: Schematic representation of the used constructs.

16

17 **Figure 4. Silencing of *OGT* affects HCV morphogenesis and infectivity.** (A) Analysis of
18 HCV infectivity. Huh7.5.1 cells were transfected with siCtrl, a pool of siRNA against *OGT* or
19 ApoE as a loss-of-function control to perturb HCV assembly, prior to infection with HCVcc
20 (Jc1) two days later (entry and replication). Mock-transfected cells were used as control
21 (Ctrl). After another 48h, intra- and extracellular HCVcc particles were used to infect naïve
22 Huh7.5.1 cells (assembly and infectivity). Virus supernatants of Huh7.5.1 cells were assayed
23 by (left) endpoint dilution assay (TCID50). Intra- and extracellular HCV RNA was purified and
24 analyzed by RT-qPCR to calculate (right) the specific infectivity (TCID50/RNA). Data are
25 expressed as mean percentage as compared to control ± s.d. Results are from four
26 independent experiments in triplicate. The dashed line indicates values from control-
27 transfected cells set at 100%. Statistics: *, *p*-value < 0.05, Mann-Whitney test. (B) Genotype-
28 independent effect of *OGT* on HCV infection. Huh7.5.1 cells were transfected with siCtrl or

1 siOGT prior to infection with HCVcc JcR2a (genotype 2a), H77R2a (genotype 1a) or
2 Con1R2a (genotype 1b). Experiments were carried out and analyzed as described in A. Data
3 are expressed as mean percentage of Renilla luciferase activity as compared to control \pm s.d.
4 Results are from three independent experiments in quadruplicate. The dashed line indicates
5 values from control-transfected cells set at 100%. Statistics: *, p -value < 0.05, Mann-Whitney
6 test. (C) Activity of OGT/OGA inhibitors on O-GlcNAcylation. The activity of Ac₄5S-GlcNAc
7 (OGT inhibitor) or Thiamet G (OGA inhibitor) on O-GlcNAcylation of proteins in Huh7.5.1
8 cells was demonstrated by Western blot as described in Supplementary Methods. (D) Effect
9 of O-GlcNAcylation on HCV infectivity. Huh7.5.1 cells were electroporated with HCVcc
10 (JcR2a), prior to treatment with increasing concentrations of Ac₄5S-GlcNAc (OGT inhibitor,
11 left) or Thiamet G (OGA inhibitor, right) 4h later. After 96h, supernatants were transferred
12 onto naïve Huh7.5.1 cells and electroporated cells were lysed to determine luciferase
13 activity. Luciferase activity in infected Huh7.5.1 cells was assessed 72h later. Data are
14 expressed as mean percentage as compared to control \pm s.d. Results are from three
15 independent experiments in quadruplicate. The dashed line indicates values from vehicle-
16 treated cells set at 100%. Statistics: *, p -value < 0.05, Mann-Whitney test.

17

18 **Figure 5. Silencing of OGT modulates HCVcc biophysical properties.** (A) Separation of
19 HCVcc by iodixanol density gradient ultracentrifugation. HCVcc were produced in non-
20 targeting siRNA control- or siOGT-transfected Huh7.5.1 cells. After overlaying HCVcc
21 (JcR2A) on a 4%-40% iodixanol step gradient and ultracentrifugation for 16h, fractions of
22 HCV particles were used to infect naïve Huh7.5.1 cells in order to determine TCID₅₀. HCV
23 RNA of each fraction was purified and analyzed by RT-qPCR. Data are expressed as mean \pm
24 s.d. from three independent experiments. (B) Specific infectivity (TCID₅₀/RNA) was
25 calculated and the density was determined by weighting each fraction. Specific infectivity of
26 each fraction is expressed as fold change as compared to the total infectivity of the control.
27 Data are expressed as mean \pm s.d. from three independent experiments. (C-D) ApoE and
28 ApoB concentrations in the individual fractions were determined by ELISA. The dashed lines

1 indicate limits of quantification of the assays. Data are expressed as mean \pm s.d. from three
2 independent experiments.

3

4 **Figure 6. Silencing of *OGT* increases the size of HCVcc.** (A) Representative pictures of
5 HCV particles generated in Huh7.5.1 cells transfected with non-targeting siRNA (siCtrl) or
6 siOGT. (B-F) Comparative analysis of particle size distribution for immunocapture (IC) from
7 HCV particles produced in Huh7.5.1 cells transfected with siCtrl or siOGT prior to infection
8 with HCVcc (JcR2a) following sucrose-cushion purification (B) or iodixanol gradient
9 fractionation (C-F) of HCVcc. HCVcc were transferred via anti-E2 antibody AR3A on electron
10 microscopy (EM) grids through IC. Particle size distribution was assessed from a series of
11 randomly acquired electron micrographs with Image-J software (NIH). Results from one of
12 three (A-B) or two (C-F) independent experiments are shown. Black lines: size distribution of
13 immunocaptured HCVcc produced in siCtrl-transfected cells. Grey lines: size distribution of
14 immunocaptured HCVcc produced in siOGT-transfected cells.

15

16 **Figure 7. *OGT* expression increases in HCC.** (A-B) Huh7.5.1 cells were infected with HCV
17 (JcR2a). After 72h, RNA and proteins were purified, and *OGT* expression analyzed by RT-
18 qPCR and Western blot. (A) Percentage of *OGT* mRNA expression relative to uninfected
19 Huh7.5.1 cells (Ctrl). Results are presented as mean \pm s.d. from three independent
20 experiments in duplicate. The dashed line indicates values from uninfected Huh7.5.1 cells
21 set at 100%. Statistics: *, p -value < 0.05, Mann-Whitney test. (B) *OGT* protein expression.
22 Left: percentage of *OGT* protein expression relative to uninfected Huh7.5.1 cells (Ctrl)
23 following quantification of Western blots as described in Supplementary Methods. Results
24 are presented as mean \pm s.d. from three independent experiments. The dashed line
25 indicates values from uninfected Huh7.5.1 cells set at 100%. Statistics: *, p -value < 0.05,
26 Mann-Whitney test. Right: representative Western blot analysis of *OGT* and actin. (C) *OGT*
27 expression and viral load in liver tissue from 22 HCV-infected patients and 6 patients not
28 infected with HCV and normal histology (see Supplementary Material and Methods).

1 Spearman correlation: $\rho = 0.06004019$, $p\text{-value} = 0.77$. (D-E) OGT expression in liver
2 tissue from 22 HCV-infected patients and 6 patients not infected with HCV and normal
3 histology according to fibrosis (D) or activity (E) scores (see Supplementary Material and
4 Methods). Wilcoxon test: F1 vs F0 $p\text{-value} = 0,38$; F2 vs F0 $p\text{-value} = 0,18$; F3 vs F0 $p\text{-value}$
5 $= 0,43$; F4 vs F0 $p\text{-value} = 0,17$; A1 vs A0 $p\text{-value} = 0,28$; A2 vs A0 $p\text{-value} = 0,23$; A3 vs A0
6 $p\text{-value} = 0,09$. (F) OGT expression in tumor (HCC) and non-tumor (Ctrl) liver tissue from
7 HCV-infected patients (34 tumor samples including 5 paired tumor/non-tumor samples),
8 hepatitis B virus (HBV)-infected patients (76 tumor samples including 7 paired tumor/non-
9 tumor samples), patients with alcoholic liver disease (ALD) (72 tumor samples including 8
10 paired tumor/non-tumor samples) and patients with non-alcoholic fatty liver disease (NAFLD)
11 (11 tumor samples including 2 paired tumor/non-tumor samples) as described in
12 Supplementary Methods. *, $p\text{-value} < 0.05$, Wilcoxon test.

13

1 **Table 1. Computational analysis of miR-501-3p and miR-619-3p targets and pathway**
 2 **enrichment.**

miRNA ID	Target gene symbol	Pathway or network	
miR-501-3p	<i>MEF2A; PPP3CA; PPP3CC</i>	<i>Calcium signaling</i>	
	<i>HMGCS1</i>	<i>Cholesterol biosynthesis</i>	
	<i>AFF4; CHMP1B; CUX1; DCLK1;</i> <i>LMX1A; PTBP2; RBMS1; RC3H1;</i> <i>SCN2A; SEC63; ZFH4</i>	Inflammatory response, dermatological diseases and conditions, inflammatory disease	
	<i>CDK6; CSDE1; GLI2; HOXD10;</i> <i>LSM5; MEF2A; MYCN; OGT;</i> <i>PPP2R2C; PPP2R5E; SEMA3C;</i> <i>TFDP2</i>	Cellular development, nervous system development and function; organ morphology	
	<i>CIT; COL10A1; FNBP1L; GAN;</i> <i>HERC1; KPNA4; NONO; SHPRH;</i> <i>STRN; TARDBP; UBE2H; USP37</i>	Cell death and survival; cellular compromise; free radical scavenging	
	<i>ATXN1; CBLL1; CEBPA; DCC;</i> <i>PEX5L; RCC2; RNF144A; ZC3H12C</i>	Cell morphology, cellular assembly and organization; cellular function and maintenance	
	miR-619-3p	<i>RUNX1T1; SMAD3</i>	<i>Adipocyte biogenesis</i>
		<i>FOXG1; GPBP1; MID1; MKL2; MSI1;</i> <i>PCBP2; WDFY3</i>	Cell cycle; organismal injury and abnormalities; cancer
		<i>ACVR2B; DCX; ESRRG; MAPK9;</i> <i>OGT; PCBP1; PDE3B; SMAD3;</i> <i>SMARCC1; TGFB3; PAPOLA</i>	Carbohydrate metabolism, energy production; small molecule biochemistry
		<i>RUNX1T1; SHANK2; SLC35D1</i>	Gene expression, lipid metabolism, small molecule biochemistry

3

4

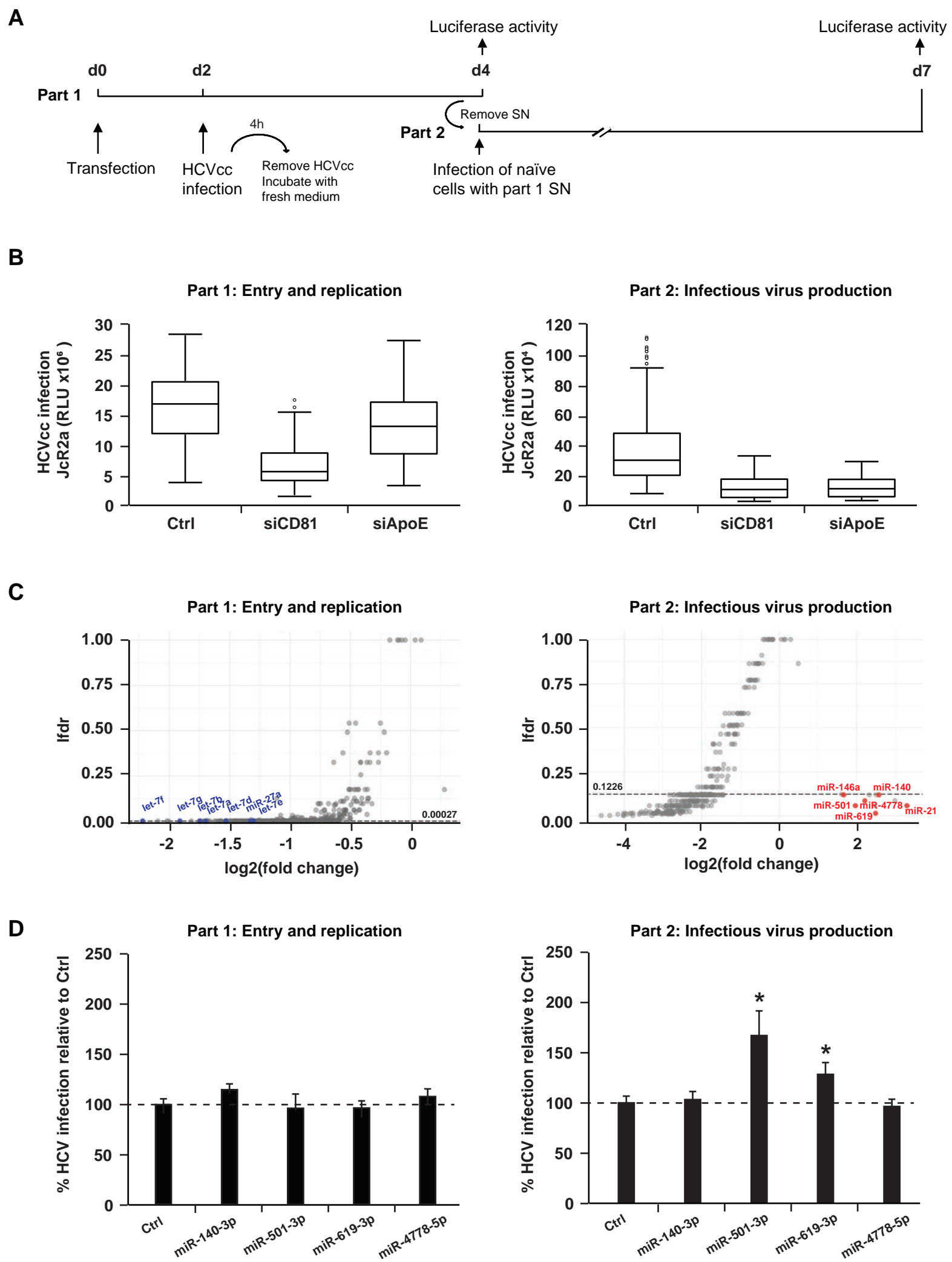
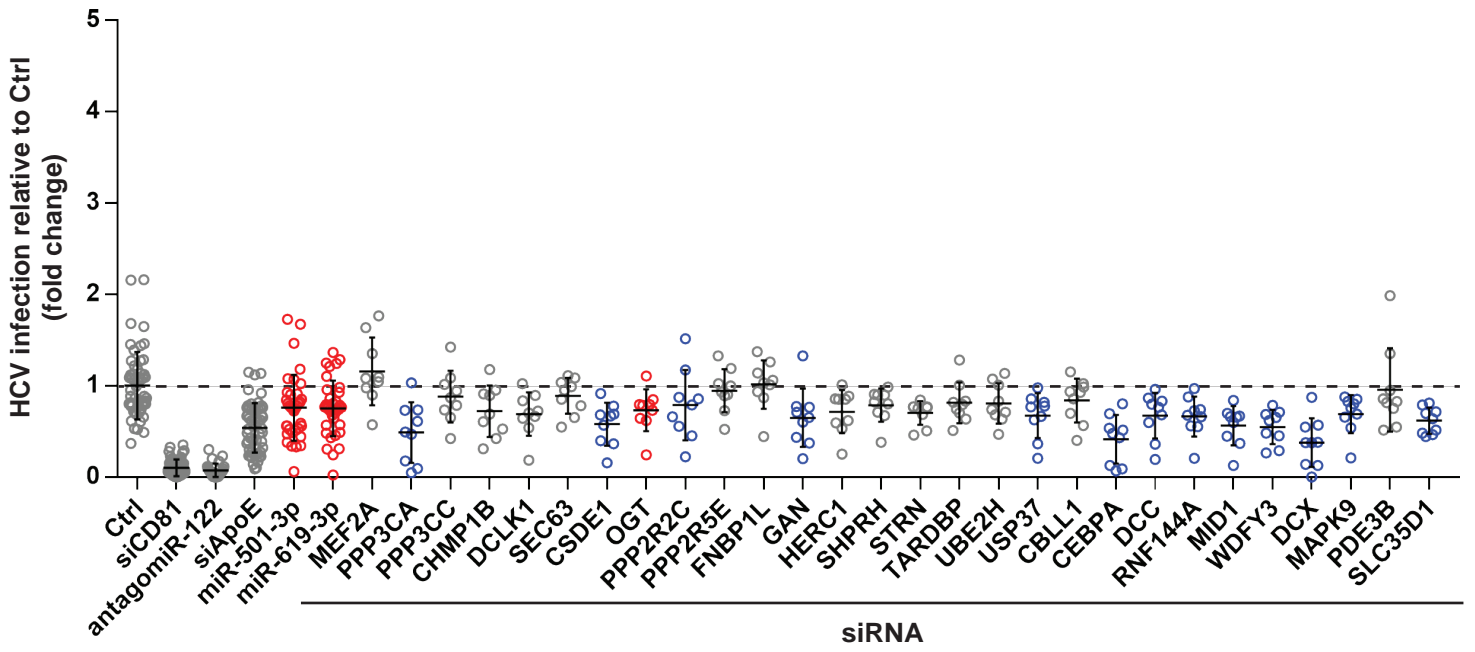


Figure 1

Part 1: Entry and replication



Part 2: Infectious virus production

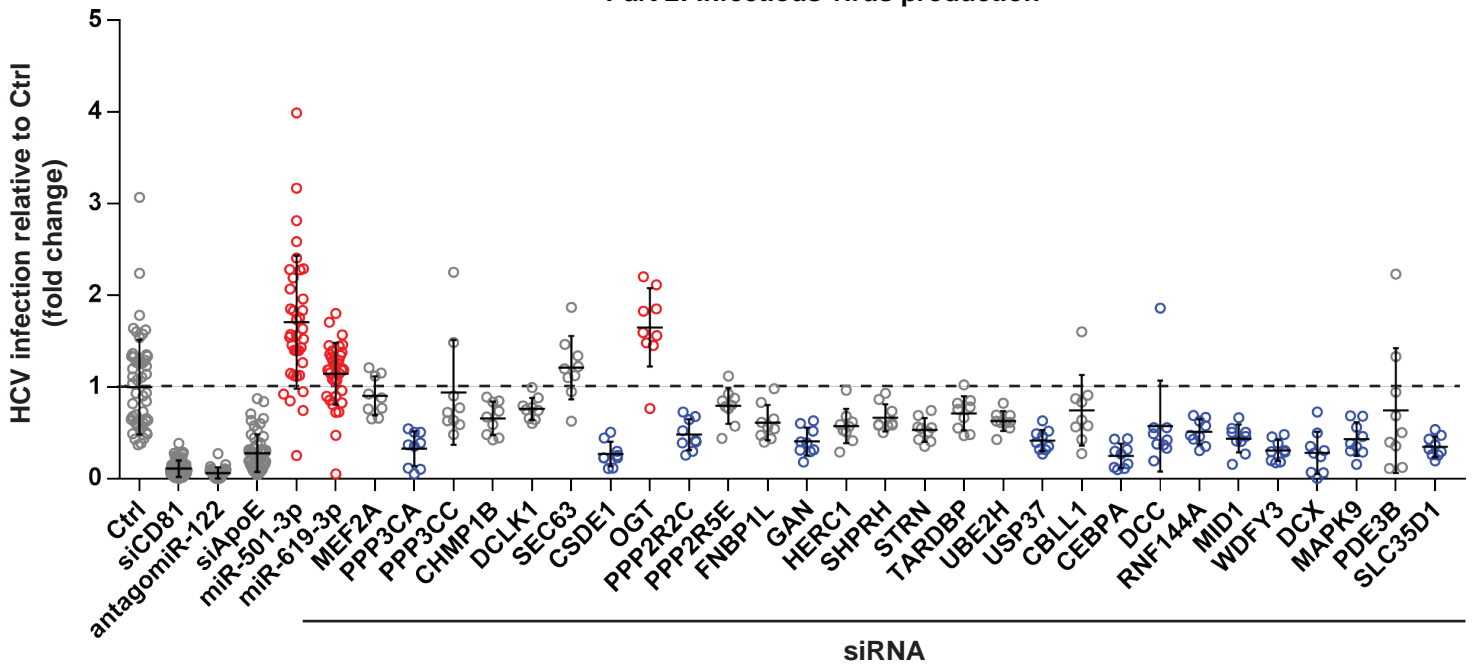
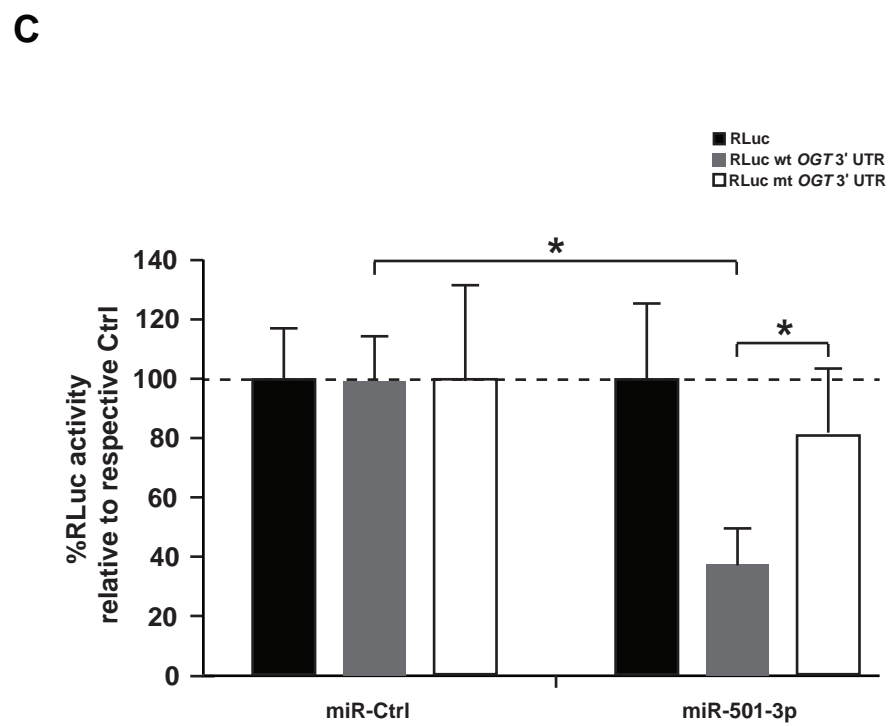
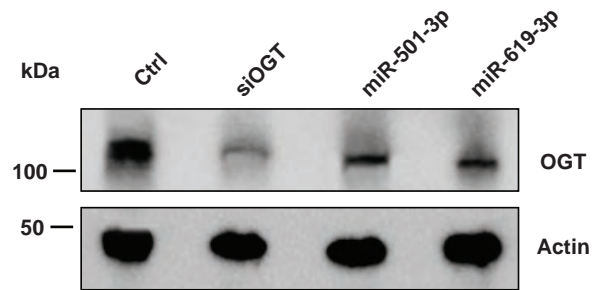
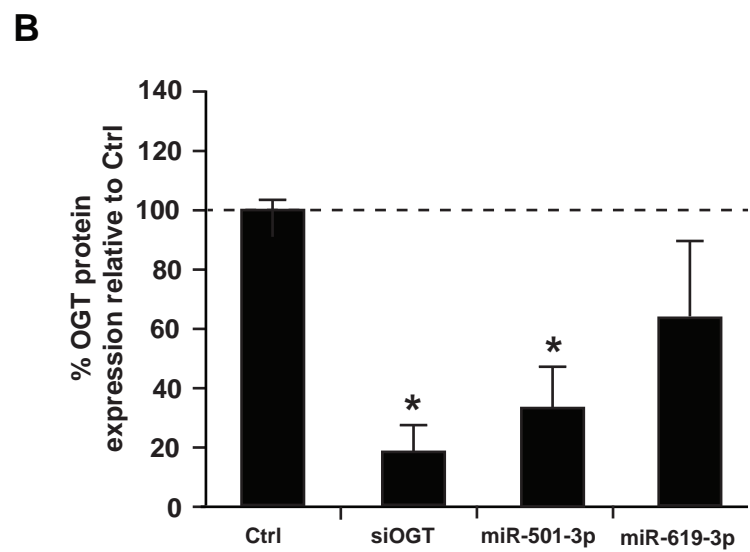
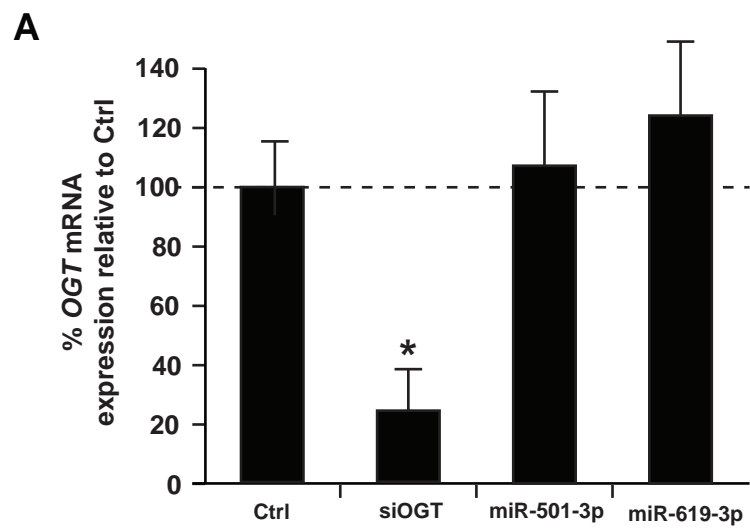
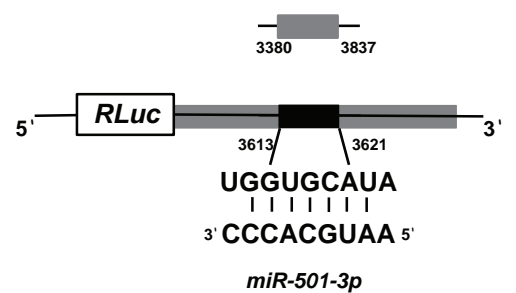


Figure 2



Wild-type sense *OGT* 3' UTR fragment



Mutant sense *OGT* 3' UTR fragment

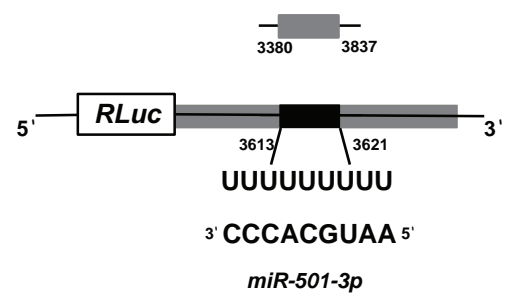
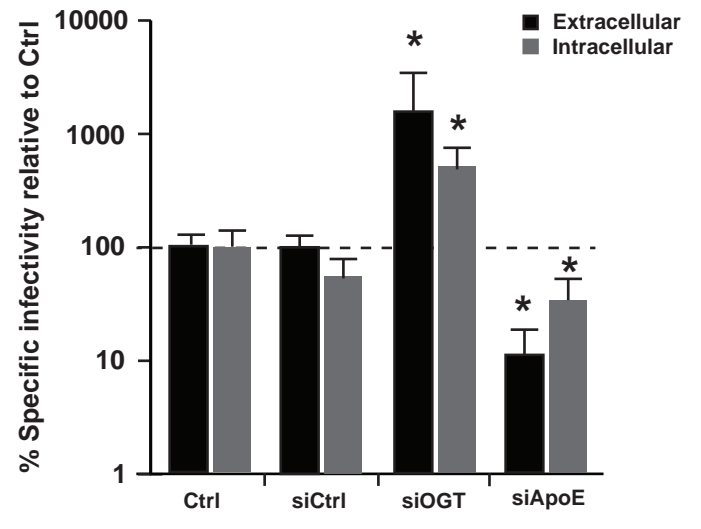
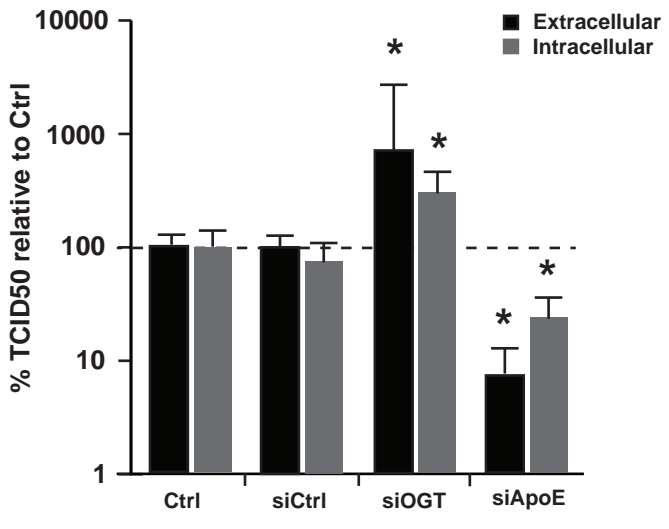
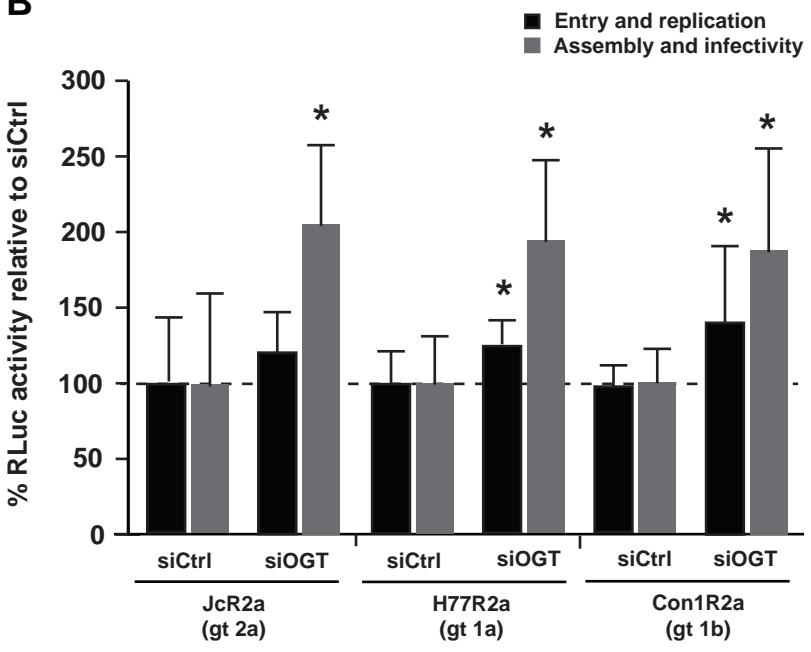
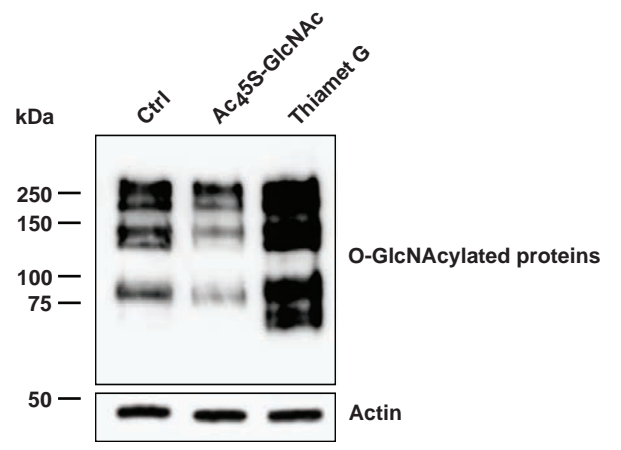
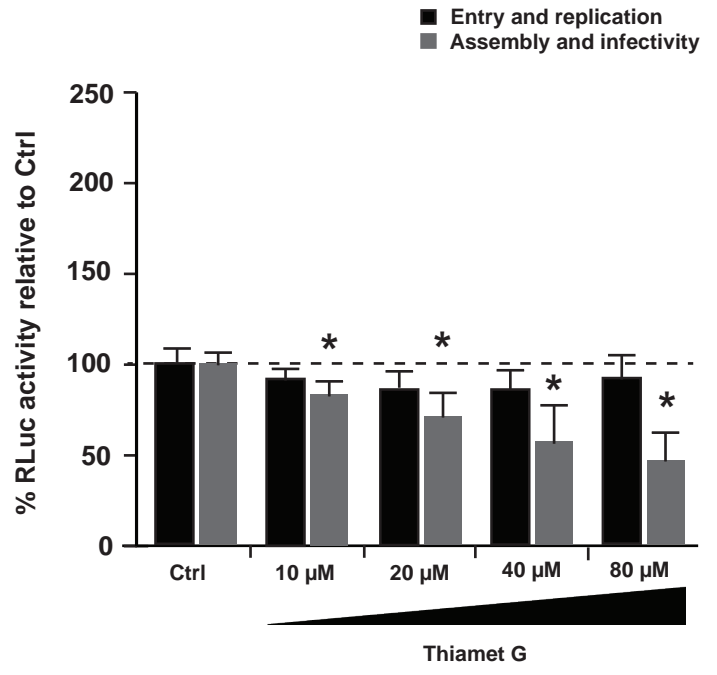
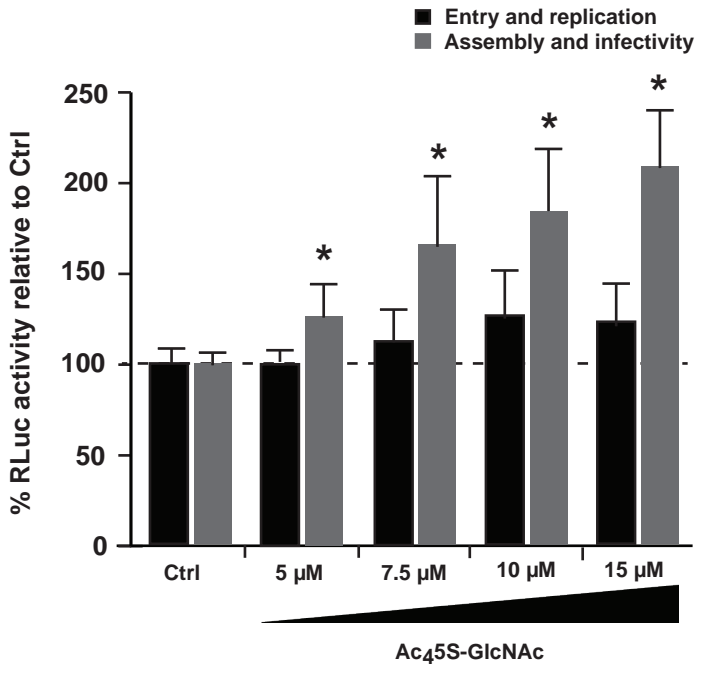


Figure 3

A**B****C****D****Figure 4**

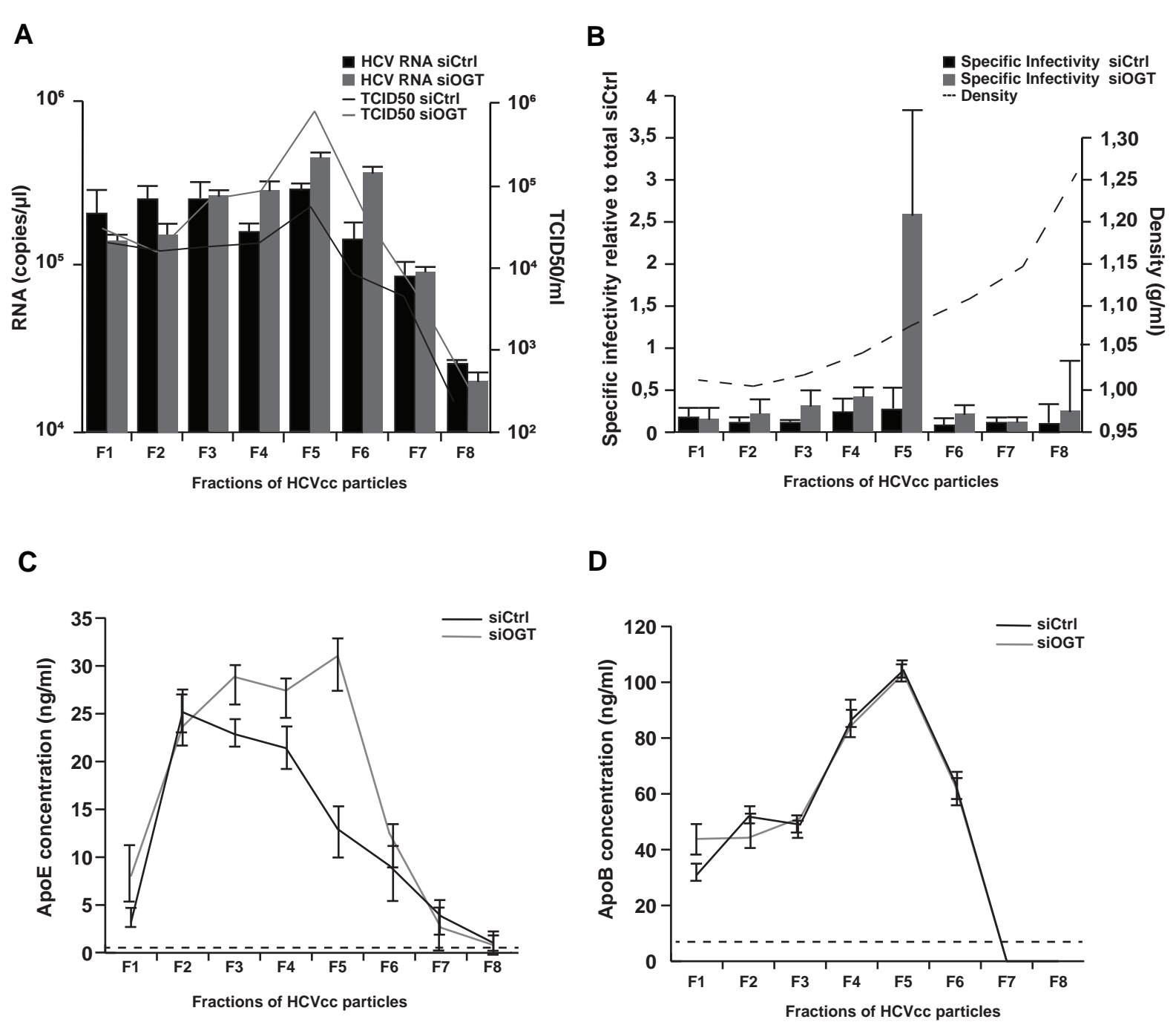


Figure 5

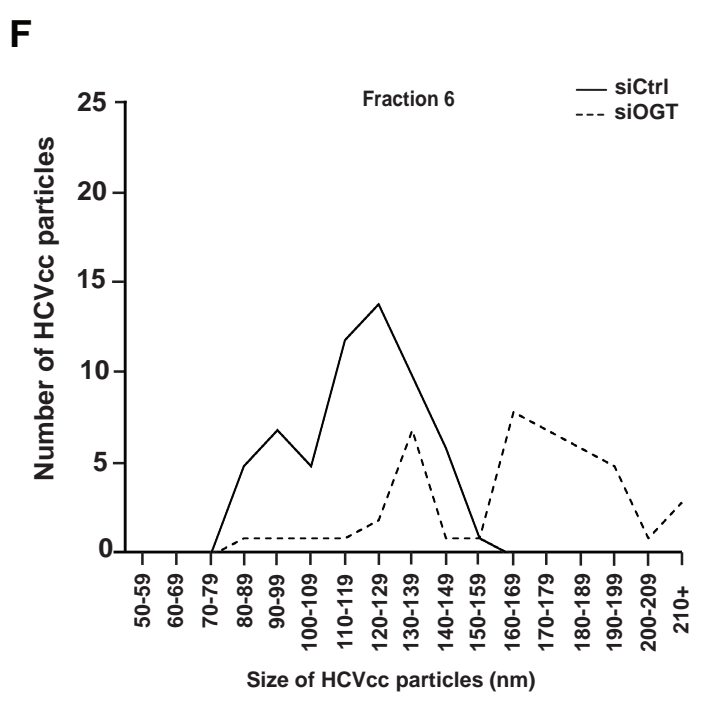
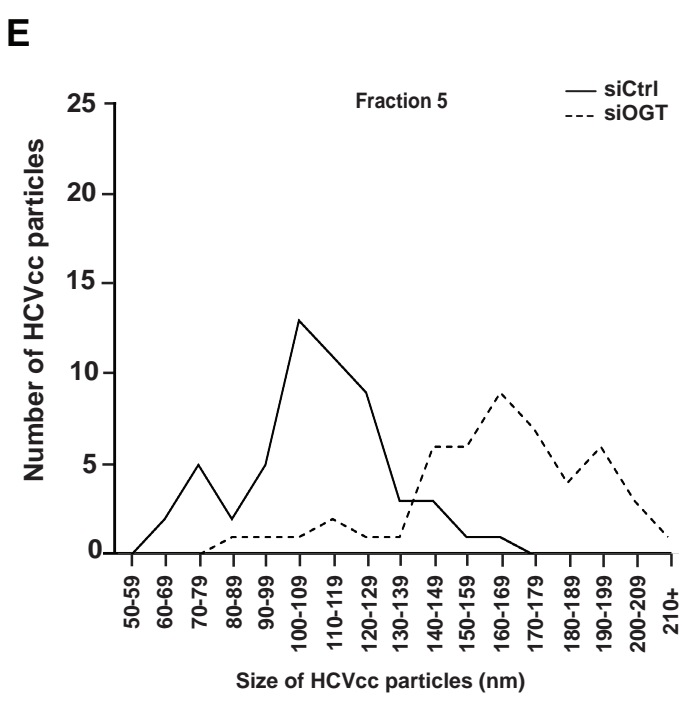
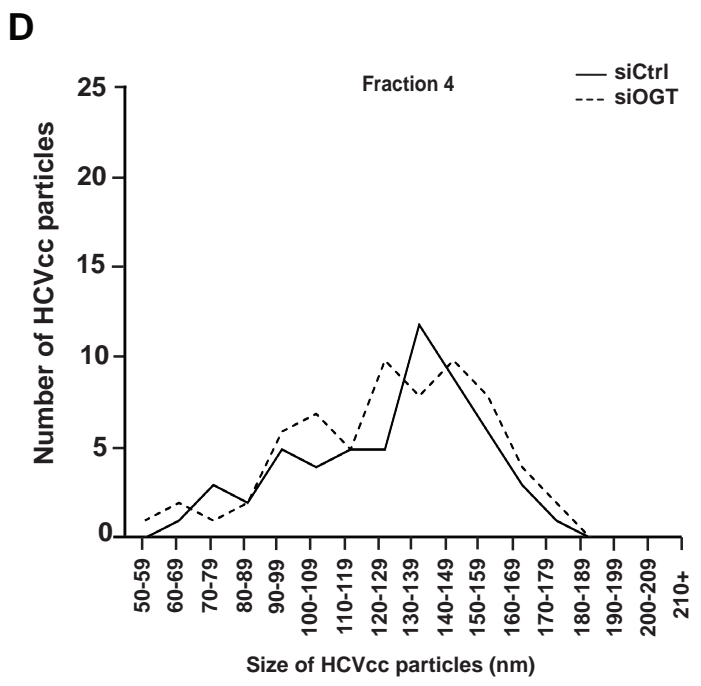
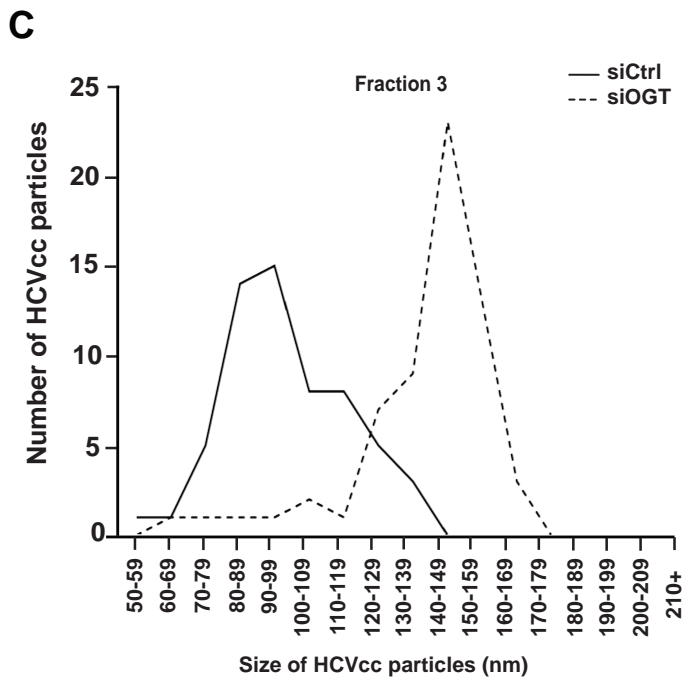
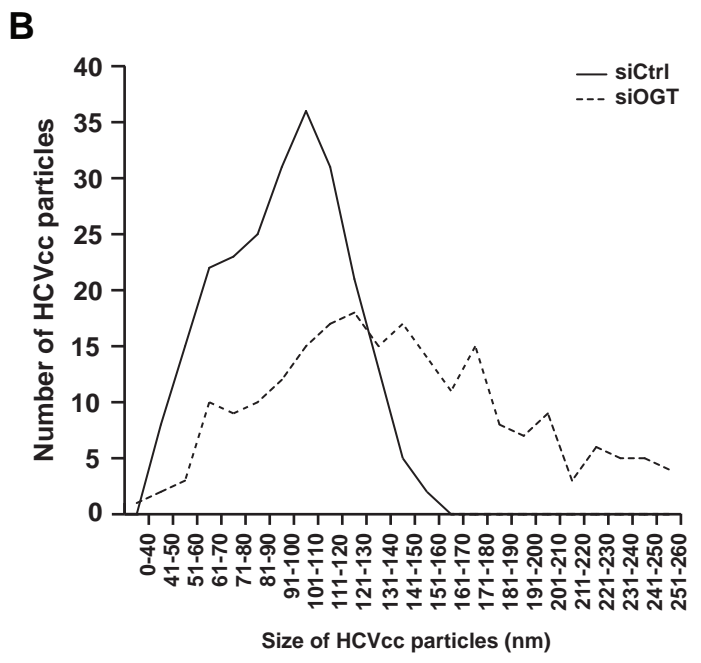
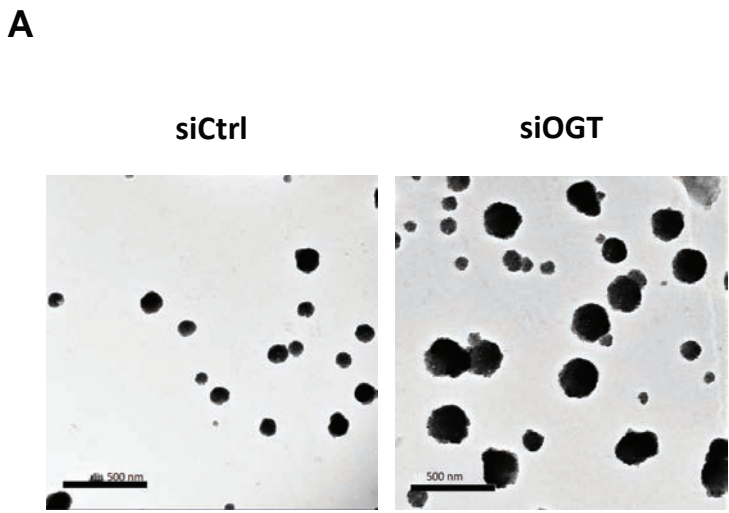


Figure 6

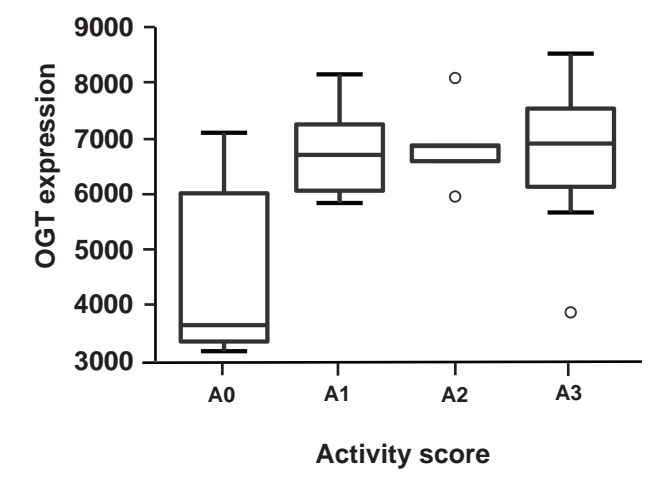
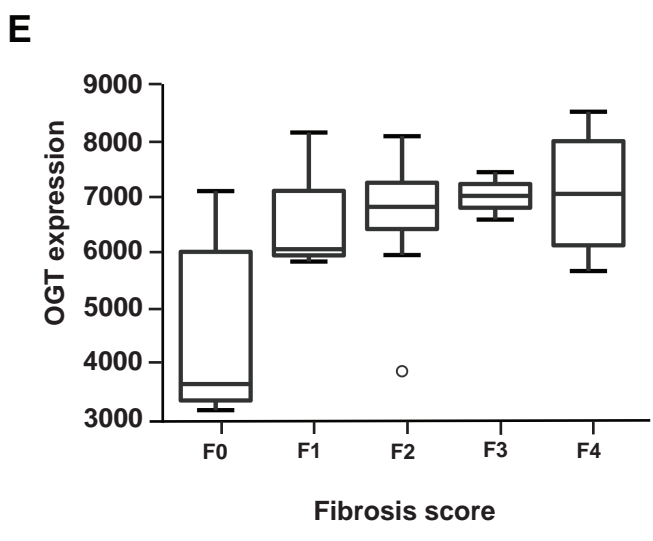
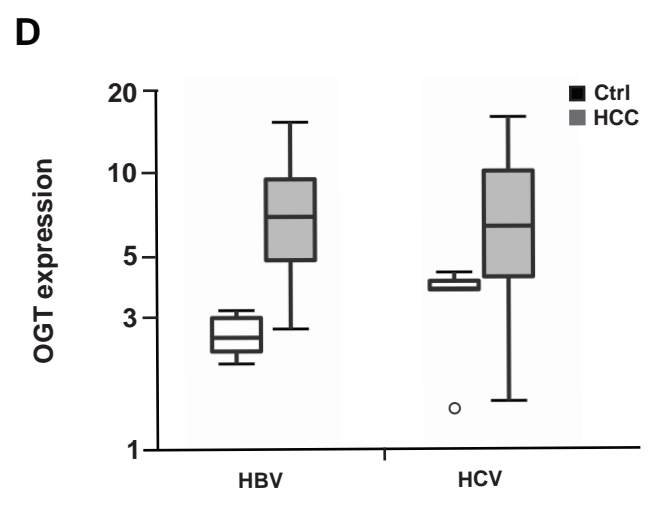
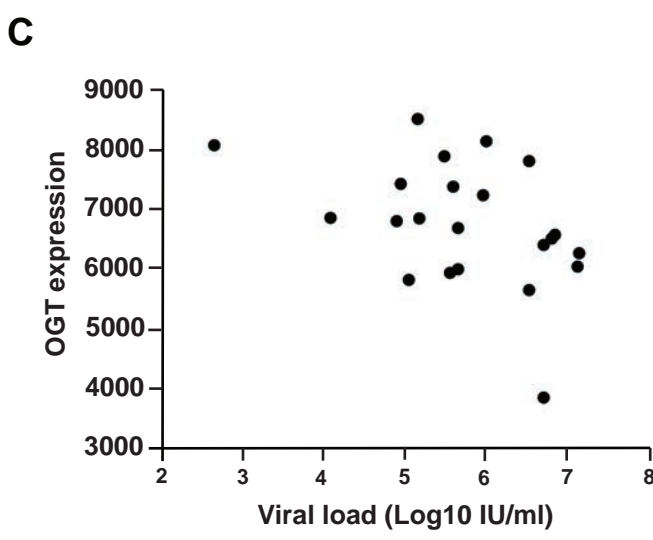
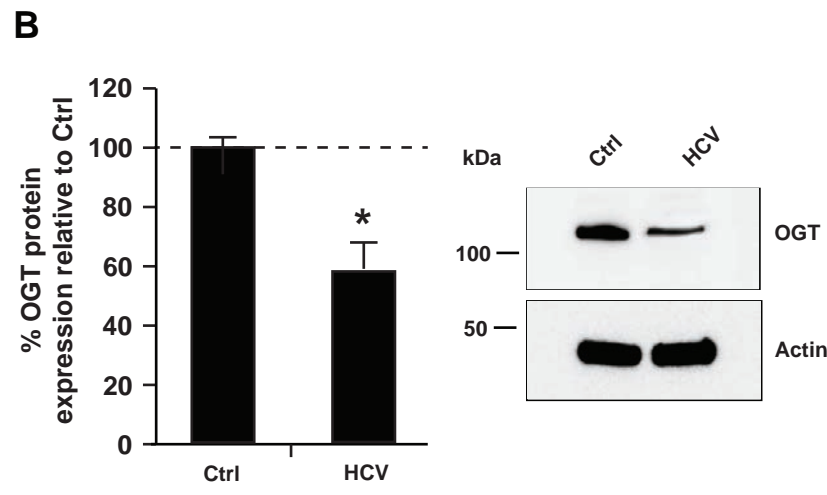
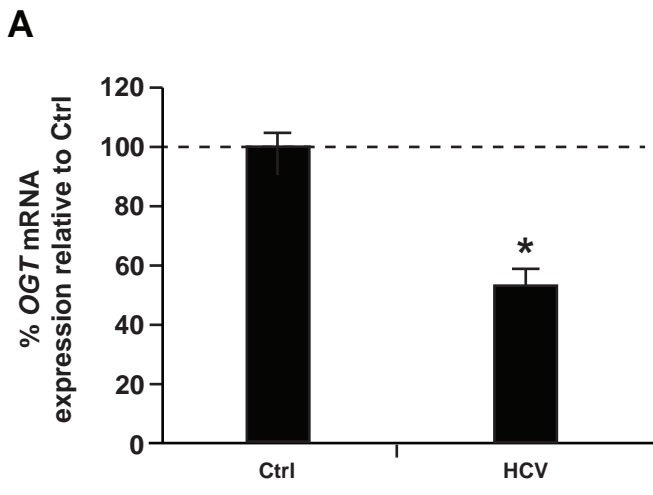


Figure 7

Supplementary Information

A functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as a host factor modulating hepatitis C virus morphogenesis

Katharina Herzog^{1,2*}, Simonetta Bandiera^{1,2*}, Sophie Pernot^{1,2}, Catherine Fauvelle^{1,2}, Frank Jühling^{1,2}, Amélie Weiss^{2,3,4,5}, Anne Bull⁶, Sarah C. Durand^{1,2}, Béatrice Chane-Woon-Ming^{2,7}, Sébastien Pfeffer^{2,7}, Marion Mercey⁸, Hervé Lerat⁸, Jean-Christophe Meunier⁶, Wolfgang Raffelsberger^{2,3,4,5}, Laurent Brino^{2,3,4,5}, Thomas F. Baumert^{1,2,9,#}, Mirjam B. Zeisel^{1,2,10,#}

¹Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France; ²Université de Strasbourg, Strasbourg, France; ³Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France ; ⁴CNRS, UMR7104, Illkirch, France ; ⁵Inserm, U1258, Illkirch, France ; ⁶Inserm U1259, Faculté de Médecine, Université François Rabelais and CHRU de Tours, Tours, France ; ⁷Architecture et Réactivité de l'ARN – UPR 9002, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France; ⁸Institute for Advanced Biosciences, Centre de Recherche UGA - Inserm U1209 - CNRS UMR 5309, Grenoble, France, ⁹Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; ¹⁰Inserm, U1052, CNRS UMR 5286, Centre Léon Bérard (CLB), Cancer Research Center of Lyon (CRCL), Université de Lyon (UCBL), Lyon, France

*Authors contributed equally to this work

Supplementary Material and methods

Cells and cell culture conditions. The source and culture conditions of Huh7.5.1 cells have been described[1]. HeLa cells were purchased from ATCC and cultured in Dulbecco's modified Eagle medium (Gibco® DMEM GlutaMAX™, ThermoFisher Scientific) containing 1% sodium pyruvate as described for Huh7.5.1 cells[1].

Viruses and infectivity assays. Cell culture-derived recombinant cell culture-derived hepatitis C virus (HCVcc) Jc1 (genotype 2a/2a chimera), H77R2a (genotype 1a/2a chimera engineered for *Renilla* luciferase expression), Con1R2a (genotype 1b/2b chimera engineered for *Renilla* luciferase expression), and JcR2a (genotype 2a/2a chimera engineered for *Renilla* luciferase expression) were generated in Huh7.5.1 cells as described[1, 2, 3, 4]. HCVcc infectivity was determined by calculating the 50% tissue culture infectious dose (TCID₅₀) using anti-NS5A antibody as described[5, 6] or by assessing luciferase activity. HCVcc were used at 10⁵-10⁶ TCID₅₀/mL throughout the study. HCV RNA was purified using a QIAmp viral RNA minikit (Qiagen) and analyzed by one-step RT-qPCR using a Sensi Fast NO ROX kit (Bioline) according to the manufacturer's instructions. Standard curves were performed using 10-fold dilution series of HCV RNA.

Purification of HCVcc particles using sucrose cushion or iodixanol density gradient.

HCVcc (JcR2a) were concentrated 10-fold using a Vivaspin column (GE Healthcare). For sucrose cushion purification, HCVcc were purified by overlaying 3.5 mL of culture media on 1.5 mL of 20% sucrose, and by ultracentrifuging samples for 4h at 40,000 rpm on a SW-55 rotor (Beckman Coulter). Purified HCVcc were resuspended in 30 µL of PBS for analysis via immunocapture and electron microscopy. Density distributions of infectious HCVcc were determined by overlaying 0.5 mL culture media on a 5 mL, 4%-40% iodixanol step gradient, and ultracentrifuging samples for 16h at 40,000 rpm on a SW-55 rotor (Beckman Coulter): 625 µl fractions were carefully harvested from the top of each tube, and density was determined by weighing. Infectivity of each fraction was quantified by TCID₅₀ using anti-NS5A antibody as described[5, 6], while HCV RNA of fractions was purified and analyzed as described above. ApoB and ApoE concentrations of fractions were determined by enzyme-linked immunosorbent assay (Human Apolipoprotein B or E ELISA^{PRO} kit, Mabtech) undiluted or in a 1:50 dilution, respectively, according to the manufacturer's instructions (Mabtech).

miRNA mimics and siRNAs. Non-targeting control miRNA, miR-501-3p mimic, miR-619-3p mimic, antagomiR-122, antagomiR-501-3p, non-targeting control antagomiR, non-targeting control siRNA, siRNAs targeting *OGT*, *CD81* or *apolipoprotein E (ApoE)* and a library of 28 custom ON-TARGETplus smart pool siRNAs were purchased from Dharmacon (GE Healthcare).

miRNA expression analysis. Total RNA (100 ng) was purified from control or HCV-infected Huh7.5.1 cells using Tri reagent® (Thermo Scientific) and Direct-zol™ RNA purification kit (Zymo Research). Total RNA was first polyadenylated and reverse transcribed using a miScript II RT system (Qiagen) according to the manufacturer's instructions. The obtained cDNA was subjected to RT-qPCR using miScript SYBR Green kit (Qiagen). Primers were the mature miRNA sequence for the forward primer (Thermo Scientific) and the universal miScript primer (Qiagen) for the reverse primer. Data were analyzed by the $\Delta\Delta C_t$ method using small nucleolar RNA, C/D box 61 (SNORD61) as an endogenous reference and the non-infected samples as a calibrator[7].

Antibodies. Rabbit anti-OGT antibodies DM-17 and AL24 were purchased from Sigma or kindly provided by Dr. G. W. Hart and Dr. S. Hardivillé (Johns Hopkins University School of Medicine, Baltimore, MD)[8], respectively. Mouse anti- β -actin antibody was purchased from Abcam and mouse, rabbit or sheep HRP-conjugated secondary antibodies (A9044, A0545 and A3415, respectively) were purchased from Sigma. Sheep anti-NS5A serum for determination of TCID₅₀ was a kind gift from M. Harris[9]. Human anti-E2 (AR3A) antibody[10] for electron microscopy analysis was kindly provided by Mansun Law (SCRIPPS, California, USA).

Western blotting. OGT and actin protein expression in human cells was assessed by Western blot as described[8] with some modifications. Briefly, cells were lysed in lysis buffer no. 6 (R&D Systems) according to the manufacturer's instructions. Equal amounts of protein

(40 µg) were size-separated through a Mini PROTEAN® TGX Stain-Free™ gel electrophoresis (Bio-Rad) and transferred to PVDF membranes (Bio-Rad). Immunoblots were performed using rabbit anti-OGT (1:2000) and mouse anti-β-actin (1:1000) antibodies[8, 11]. Antigen-antibody complexes were detected by incubating the membrane with the appropriate HRP-conjugated secondary antibodies (1:5000; 1:10,000) and imaged by enhanced chemiluminescence with a ChemiDoc MP imager (Bio-Rad). Quantification of protein expression was performed using ImageLab™ 5.2.1 software (BioRad). For analysis of OGT and GAPDH expression in liver tissue from HCV transgenic (FL-N/35) or wild-type mice[12], crude protein extracts were prepared by homogenization of frozen mouse livers (50–100 µg) in tissue lysis buffer from the Ambion PARIS RNA (Thermo Scientific) and protein isolation kit, supplemented with protease inhibitors (cOmplete™ EDTA-free protease inhibitor mixture, Sigma-Aldrich) and phosphatase inhibitors (PhosSTOP™, Sigma-Aldrich), using a tissue homogenizer (MP Fast Prep24, MP Biomedicals, Santa Ana, CA) and MP Lysing Matrix A tubes. Proteins were quantified using the BCA assay (Thermo Fisher Scientific). Western blotting was performed as described above.

Immunocapture and electron microscopy analysis of viral particles. Sucrose-cushion purified or iodixanol gradient fractionated HCVcc (JcR2a) produced in cells transfected with a non-targeting siRNA control or a pool of siRNA against OGT were transferred via anti-E2 antibody AR3A on electron microscopy (EM) grids through immunocapture (IC) as described[13]. Particles were stained with uranyl acetate dihydrate and observed in a JEOL 1230 electron microscope. Series of electron micrographs were acquired at random from IC EM grids. The images were then analyzed with Image-J software, to determine the particle size distribution.

Gene expression analysis in patient-derived liver tissue. For OGT expression analysis in patient's samples, raw data were retrieved from the Gene Expression Omnibus (GSE84346) and re-analyzed by quality-trimming (cutadapt) and mapping (HISAT2) to human genome

assembly hg19. Reads mapping to Gencode v.19 genes were counted using htseq-count and normalized applying DESeq2. Activity and fibrosis scores as well as viral load were taken from the supplemental data of[14] including 22 HCV-infected patients and 6 patients not infected with HCV and normal histology who underwent liver biopsy due to unclear hepatopathy (n=4), metastasis of breast cancer (n=1), or metastasis of lung cancer (n=1) as described in[14]. To analyze OGT expression in liver tissue of patients with chronic liver disease, FPKM values and clinical data were retrieved from The Cancer Genome Atlas (TCGA, <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>). This data set includes samples from HCV-infected patients (34 tumor samples including 5 paired tumor/non-tumor samples), hepatitis B virus (HBV)-infected patients (76 tumor samples including 7 paired tumor/non-tumor samples), patients with alcoholic liver disease (ALD) (72 tumor samples including 8 paired tumor/non-tumor samples) and patients with non-alcoholic fatty liver disease (NAFLD) (11 tumor samples including 2 paired tumor/non-tumor samples).

Supplementary References

- 1 Zhong J, Gastaminza P, Cheng G, *et al.* Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A 2005;**102**:9294-9.
- 2 Reiss S, Rebhan I, Backes P, *et al.* Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. Cell Host Microbe 2011;**9**:32-45.
- 3 Da Costa D, Turek M, Felmlee DJ, *et al.* Reconstitution of the entire hepatitis C virus life cycle in non-hepatic cells. J Virol 2012;**86**:11919-25.
- 4 Fauvelle C, Felmlee DJ, Crouch E, *et al.* Apolipoprotein E Mediates Evasion From Hepatitis C Virus Neutralizing Antibodies. Gastroenterology 2016;**150**:206-17 e4.
- 5 Lindenbach BD, Evans MJ, Syder AJ, *et al.* Complete replication of hepatitis C virus in cell culture. Science 2005;**309**:623-6.

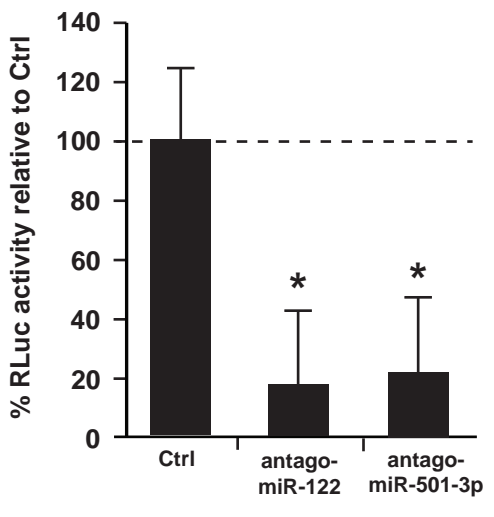
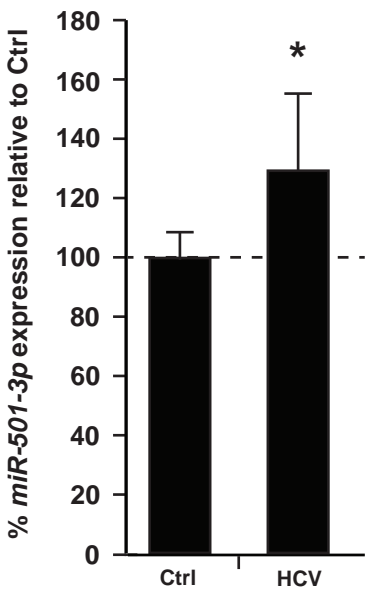
- 6 Bandiera S, Pernot S, El Saghire H, *et al.* Hepatitis C Virus-Induced Upregulation of MicroRNA miR-146a-5p in Hepatocytes Promotes Viral Infection and Deregulates Metabolic Pathways Associated with Liver Disease Pathogenesis. *J Virol* 2016;**90**:6387-400.
- 7 Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008;**3**:1101-8.
- 8 Iyer SP, Akimoto Y, Hart GW. Identification and cloning of a novel family of coiled-coil domain proteins that interact with O-GlcNAc transferase. *J Biol Chem* 2003;**278**:5399-409.
- 9 Macdonald A, Crowder K, Street A, *et al.* The hepatitis C virus non-structural NS5A protein inhibits activating protein-1 function by perturbing ras-ERK pathway signaling. *J Biol Chem* 2003;**278**:17775-84.
- 10 Giang E, Dorner M, Prentoe JC, *et al.* Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. *Proc Natl Acad Sci U S A* 2012;**109**:6205-10.
- 11 Verrier ER, Colpitts CC, Bach C, *et al.* A targeted functional RNA interference screen uncovers glypican 5 as an entry factor for hepatitis B and D viruses. *Hepatology* 2016;**63**:35-48.
- 12 Lerat H, Honda M, Beard MR, *et al.* Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. *Gastroenterology* 2002;**122**:352-65.
- 13 Piver E, Boyer A, Gaillard J, *et al.* Ultrastructural organisation of HCV from the bloodstream of infected patients revealed by electron microscopy after specific immunocapture. *Gut* 2017;**66**:1487-95.
- 14 Boldanova T, Suslov A, Heim MH, *et al.* Transcriptional response to hepatitis C virus infection and interferon-alpha treatment in the human liver. *EMBO Mol Med* 2017;**9**:816-34.

Supplementary figure legends

Figure S1. (A) Effect of miR-501-3p inhibition on HCV infectivity. Huh7.5.1 cells were transfected with control antagomiR (Ctrl), antagomiR-122 as loss-of-function control to perturb

HCV replication and antagomiR-501-3p, prior to infection with HCVcc (JcR2a) according to the two-step protocol depicted in Fig. 1A. After 48h, supernatants were transferred onto naive Huh7.5.1 cells. After 72h, Renilla Luciferase activity of infected Huh7.5.1 cells was determined. Data are expressed as mean percentage as compared to Ctrl \pm s.d. Results are from four independent experiments in quadruplicate. The dashed line indicates values from vehicle-treated cells set at 100%. Statistics: *, p -value < 0.05, Mann-Whitney test. (B) miR-501-3p expression upon HCV infection. Huh7.5.1 cells were infected with HCVcc (JcR2a). After 72h, RNA was purified and miR-501-3p expression analyzed by RT-qPCR. Percentage of miR-501-3p expression relative to uninfected Huh7.5.1 cells (Ctrl). Results are presented as mean \pm s.d. from three independent experiments in duplicate. The dashed line indicates values from uninfected Huh7.5.1 cells set at 100%. Statistics: *, p -value < 0.05, Mann-Whitney test.

Supplementary Table 1. A genome-wide miRNA mimic screen identifies cellular miRNAs modulating HCV infection. Log₂(FC), l_{fdr} and effect on HCV infection in part 1 and part 2 of the screen are shown for the individual miRNAs of the miRNA mimic library. In red: proviral effect, in blue: antiviral effect. FC: fold change, l_{fdr}: local false discovery rate

A**B****Figure S1**

miRNA ID	Mature Sanger ID	Library ID	Mature Sequence	Part 1			Part 2		
				Log2(FC)	lfr	Effect on HCV	Log2(FC)	lfr	Effect on HCV
hsa-let-7a	MIMAT0000062	C-300473-05	UGAGGUAGUAGGUUGUAUAGUU	-1.70171935969617	3.9917e-05	TRUE	-3.28400225976063	0.0018396	TRUE
hsa-let-7b	MIMAT0000063	C-300476-05	UGAGGUAGUAGGUUGUGUGUU	-1.74684091191792	8.3715e-05	TRUE	-3.61453853652595	0.009387	TRUE
hsa-let-7d	MIMAT0000065	C-300478-07	AGAGGUAGUAGGUUGCAUAGUU	-1.53075932976348	0.00023985	TRUE	-2.74632167025162	0.057617	TRUE
hsa-let-7e	MIMAT0000066	C-300479-05	UGAGGUAGGAGGUUGUAUAGUU	-1.30271875995011	0.0014577	FALSE	-1.90993819018051	0.081661	TRUE
hsa-let-7f	MIMAT0000067	C-300480-05	UGAGGUAGUAGAUUGUAUAGUU	-2.22178435619614	6.7884e-06	TRUE	-3.76872186786763	0.014453	TRUE
hsa-let-7g	MIMAT0000414	C-300583-05	UGAGGUAGUAGUUUGUACAGUU	-1.91269526615093	6.7884e-06	TRUE	-2.71878759599202	0.057617	TRUE
hsa-miR-101	MIMAT0000099	C-300518-07	UACAGUACUGUGUAACUGAA	-0.886376348041933	0.0030028	FALSE	-1.9796810124946	0.11887	TRUE
hsa-miR-103-as	MIMAT0007402	C-301453-00	UCAUAGCCUGUACAAUGCUGCU	-1.11208100417218	0.0074787	FALSE	-2.91287954813545	0.057617	TRUE
hsa-miR-106a*	MIMAT0004517	C-301159-01	CUGCAAUGUAAGCACUUCUAC	-1.32520114049511	3.9917e-05	TRUE	-2.3773966141336	0.11887	TRUE
hsa-miR-1178	MIMAT0005823	C-301319-00	UUGCUCACUGUUCUCCCUAG	-1.22556728667978	0.00061109	FALSE	-2.3126959903038	0.08542	TRUE
hsa-miR-1178-5p	MIMAT0022940	C-301922-00	CAGGGUCAGCUGAGCAUG	-1.16672245017752	0.00026644	TRUE	-0.780775920183507	0.72813	FALSE
hsa-miR-1185	MIMAT0005798	C-301317-00	AGAGGAUACCCUUUGUAUGUU	-1.2315368528283	8.3715e-05	TRUE	-0.721316088104541	0.76975	FALSE
hsa-miR-1200	MIMAT0005863	C-301326-00	CUCCUGAGCCAUUCUGAGCCUC	-1.24866404694154	0.00054279	FALSE	-2.09711908610931	0.08542	TRUE
hsa-miR-1205	MIMAT0005869	C-301331-00	UCUGCAGGGUUUGCUUUGAG	-0.801511472736558	0.0053924	FALSE	-3.77846481724539	0.0018396	TRUE
hsa-miR-1207-3p	MIMAT0005872	C-301334-00	UCAGCUGGCCUCAUUUC	-1.10040811819955	0.00013963	TRUE	-1.69117584901668	0.26604	FALSE
hsa-miR-122	MIMAT0000421	C-300591-05	UGGAGUAGCAAUGGUUUUG	-0.7030591171691	0.040577	FALSE	-3.13425305602985	0.028781	TRUE
hsa-miR-122*	MIMAT0004590	C-301046-01	AACGCCAUUAUCACACUAAUA	-0.557131436052541	0.037103	FALSE	-1.83404561163316	0.11887	TRUE
hsa-miR-1226	MIMAT0005577	C-301284-01	UCACCCAGCCUGUUCUCCUAG	-0.741744822716559	0.0099219	FALSE	-1.89688153334356	0.11887	TRUE
hsa-miR-1228*	MIMAT0005582	C-301287-01	GUGCGCGGGGCGAGGUGUG	-0.947568053691086	0.0049772	FALSE	-2.29154418859234	0.11887	TRUE
hsa-miR-1237-5p	MIMAT0022946	C-302618-00	CGGGGGCGGGCCGAACGCGG	-0.956259568604456	0.021994	FALSE	-2.34546898601693	0.057617	TRUE
hsa-miR-1244	MIMAT0005896	C-301865-00	AAGUAGUUGGUUUGUAUGAGAUGGU	-1.17945761818024	6.1693e-05	TRUE	-0.989861012597079	0.5811	FALSE
hsa-miR-1245b-3p	MIMAT0019951	C-302405-00	UCAGAUAGUCAAAGGCCUUAUA	-0.414846848981959	0.32544	FALSE	-2.27819747964432	0.081661	TRUE
hsa-miR-1255b-2-3p	MIMAT0022725	C-301882-00	AACCACUUUCUUCUCUCAUCCA	-0.427268737714942	0.061384	FALSE	-2.58892190360625	0.023579	TRUE
hsa-miR-1258	MIMAT0005909	C-301384-00	AGUUAGGAUUAAGCUGGGAA	-1.57210875214914	1.5894e-05	TRUE	-1.63979276673705	0.36109	FALSE
hsa-miR-125b-2*	MIMAT0004603	C-301061-01	UCACAAGUCAGGCUCUUGGGAC	-0.858902068537019	0.0052694	FALSE	-1.87406855538924	0.11887	TRUE
hsa-miR-1260b	MIMAT0015041	C-301716-00	AUCCACCACUGCCACCAU	-0.476317862532308	0.10991	FALSE	2.46099391534969	0.057617	TRUE
hsa-miR-1270	MIMAT0005924	C-301867-00	CUGGAGAUUGGAAGACUGUGU	-1.23553336541613	3.9917e-05	TRUE	-2.24160380684469	0.11887	TRUE
hsa-miR-1277	MIMAT0005933	C-301411-00	UACGUAGAUUAUUAUGUAUUUU	-0.745198002045239	0.0074787	FALSE	-3.58615793633136	0.014453	TRUE
hsa-miR-1278	MIMAT0005936	C-301417-00	UAGUACUGUGCAUAUCAUCUAU	-0.494528345361641	0.089234	FALSE	1.60846287059147	0.12226	TRUE
hsa-miR-1283	MIMAT0005799	C-301315-00	UCUACAAAGGAAAGCGCUUUUCU	-0.538161112092319	0.061384	FALSE	-2.67520055202511	0.08542	TRUE
hsa-miR-1284	MIMAT0005941	C-301423-00	UCUAUACAGACCUGGCCUUUC	-0.412436737654723	0.099257	FALSE	-2.28002191497783	0.11527	TRUE
hsa-miR-1287	MIMAT0005878	C-301341-00	UGCUGGAUCAGGUGGUUCGAGUC	-1.20683701032783	0.00023985	TRUE	-1.98393331688063	0.08542	TRUE
hsa-miR-1288	MIMAT0005942	C-301424-00	UGGACUGCCUGAUCUGGAGA	-0.704889827052788	0.0021076	FALSE	-2.89790974514386	0.057617	TRUE
hsa-miR-129-3p	MIMAT0004605	C-301063-01	AAGCCUUACCCCAAAAAGCAU	-0.999602920325089	0.0021076	FALSE	-1.88681635888787	0.11887	TRUE
hsa-miR-1291	MIMAT0005881	C-301345-00	UGGCCUUGACUGAAGCCAGCAGU	-1.21284220826454	0.00061109	FALSE	-2.02374608043906	0.11887	TRUE
hsa-miR-1293	MIMAT0005883	C-301347-00	UGGGUGGUCUGGAGAUUUGUC	-1.39687224389743	0.00026644	TRUE	-2.31295737423138	0.057617	TRUE
hsa-miR-1294	MIMAT0005884	C-301348-00	UGUGAGGUUGGCAUUGUGUCU	-1.44051810095446	8.3715e-05	TRUE	-1.95652126826796	0.057617	TRUE
hsa-miR-1295	MIMAT0005885	C-301349-00	UUAGCCCGCAGAUUGGUGUGA	-1.2640855905457	0.00087976	FALSE	-2.34227893604624	0.11887	TRUE
hsa-miR-1295b-5p	MIMAT0022293	C-302563-00	CACCCACUGUCGCGCCUAAU	-0.454374224389677	0.17522	FALSE	-2.73444344828677	0.023579	TRUE
hsa-miR-1298	MIMAT0005800	C-301318-00	UUCAUUCGGCUGCCAGAUGUA	-0.26688230187125	0.32544	FALSE	-2.44553337713544	0.11887	TRUE
hsa-miR-1302	MIMAT0005890	C-301869-00	UUGGGACAUACUUAUGCUGAAA	-1.35138412635794	3.6715e-05	TRUE	-2.60359528922327	0.057617	TRUE
hsa-miR-1302	MIMAT0005890	C-301354-00	UUGGGACAUACUUAUGCUGAAA	-1.11168868347783	0.00013793	TRUE	-1.29913904583381	0.51048	FALSE
hsa-miR-1307	MIMAT0005951	C-301434-00	ACUCGGGUGGCGUUGGUGUG	-1.12000717587297	0.00054279	FALSE	-2.10299176202445	0.057617	TRUE
hsa-miR-1307-5p	MIMAT0022727	C-301875-00	UCGACCGGACCCUGACCGGCU	-0.803481003211417	0.0030028	FALSE	-1.76491161834359	0.11887	TRUE
hsa-miR-1322	MIMAT0005953	C-301436-00	GAUGAUGCUGCUGAUGCUG	-1.23817643374983	6.1693e-05	TRUE	-1.29920754317295	0.46329	FALSE
hsa-miR-1323	MIMAT0005795	C-301311-00	UAAAACUGAGGGGCAUUUUUCU	-0.756897047236657	0.096607	FALSE	-2.43084213259459	0.11887	TRUE
hsa-miR-135b*	MIMAT0004698	C-301200-01	AUGAAGCGCUAAAAGCCAUAGGG	-1.49530939618539	0.0018747	FALSE	-2.98188718020305	0.050122	TRUE
hsa-miR-139-3p	MIMAT0004552	C-301036-03	UGGAGACGCGCCUGUUGGAGU	-1.02065377094153	0.00079918	FALSE	-2.77458369079406	0.019699	TRUE
hsa-miR-140-3p	MIMAT0004597	C-301055-01	UACCACAGGGUAGAACCACGG	-0.172675601522775	1	FALSE	2.56894688372309	0.11887	TRUE
hsa-miR-142-3p	MIMAT0000434	C-300610-03	UGUAGUUGUUCUACUUUAUGGA	-1.00474764620935	0.00023985	TRUE	-1.7577126946261	0.22566	FALSE
hsa-miR-146a	MIMAT0000449	C-300630-03	UGAGAAGUUAUCCAUUGGUGU	0.279820319960687	0.17522	FALSE	1.66479155066337	0.11887	TRUE
hsa-miR-150*	MIMAT0004610	C-301067-01	CUGGUACAGGCCUGGGGACAG	-1.46501526099695	0.00023985	TRUE	-1.19620229448666	0.40677	FALSE

hsa-miR-151b	MIMAT0010214	C-301973-00	UCGAGGAGCUCACAGUCU	-1.03668648961171	3.9917e-05	TRUE	-2.8985532020104	0.057617	TRUE
hsa-miR-182	MIMAT0000259	C-300557-07	UUUGGCCAAUUGGUAGAACUCACACU	-0.581360116993454	0.03164	FALSE	-1.81305061189634	0.11887	TRUE
hsa-miR-184	MIMAT0000454	C-300635-03	UGGACGGAGAACUGAUUAGGGU	-1.31700777618768	3.9917e-05	TRUE	-1.52851322717993	0.36109	FALSE
hsa-miR-185	MIMAT0000455	C-300636-07	UGGAGAGAAAGGCAUUCUGA	-1.59673044023595	0.00013963	TRUE	-1.74454670174015	0.1633	FALSE
hsa-miR-188*	MIMAT0004751	C-301187-01	UGCCUAAUUGCCCUUUCUGGC	-0.825568919117559	0.0027607	FALSE	-2.30862554668178	0.023579	TRUE
hsa-miR-1909	MIMAT0007883	C-301456-00	CGCAGGGCCGGGUGUCUACCG	-0.511863313449074	0.17522	FALSE	2.49877552994666	0.11887	TRUE
hsa-miR-1915*	MIMAT0007891	C-301466-00	ACCUUGCCUUGCUGCCGGGCC	-1.71935071114424	8.3715e-05	TRUE	-1.13532112712663	0.46329	FALSE
hsa-miR-196b*	MIMAT0009201	C-301305-00	UCGACAGCACGACUGCCUUC	-0.949306838412525	0.00054279	FALSE	-3.1358874675771	0.023579	TRUE
hsa-miR-19b	MIMAT0000074	C-300489-03	UGUGCAAUUGCCUUAACUGA	-1.01461439258753	0.00013793	TRUE	-2.75192926226938	0.014453	TRUE
hsa-miR-19b-1*	MIMAT0004491	C-301021-01	AGUUUUGCAGGUUUUGCAUCCAGC	-1.68388148726454	0.0030028	FALSE	-2.85695043305243	0.014453	TRUE
hsa-miR-19b-2*	MIMAT0004492	C-301139-01	AGUUUUGCAGGUUUUGCAUUUCA	-0.594068837054281	0.037103	FALSE	-2.06967686748099	0.11887	TRUE
hsa-miR-200b*	MIMAT0004571	C-301144-01	CAUCUUAUCUGGCGAGUUGCA	-1.05721574165849	0.0074787	FALSE	-4.59657299807562	0.0018396	TRUE
hsa-miR-203b-5p	MIMAT0019813	C-302277-00	UAGUGUCCUAAAACAUUUCAGA	-1.0335612225008	0.00016067	TRUE	-2.13526669981558	0.08542	TRUE
hsa-miR-208a	MIMAT0000241	C-300537-03	AUAAGACGAGCAAAAAGCUUGU	-0.823665097293502	0.0030028	FALSE	-1.54639877799123	0.11887	TRUE
hsa-miR-21	MIMAT0000076	C-300492-03	UAGCUUAUCAGACUGAUGUUGA	-0.768228630205262	0.096607	FALSE	3.29607415881261	0.057617	TRUE
hsa-miR-211-3p	MIMAT0022694	C-301905-00	GCAGGGACAGCAAAGGGGUGC	-0.812107428438748	0.0027607	FALSE	-2.21028883028492	0.08542	TRUE
hsa-miR-2114	MIMAT0011156	C-301489-00	UAGUCGCUUUCUAGAGCCGUC	-0.958796902783202	0.00016326	TRUE	-1.5768286317563	0.26604	FALSE
hsa-miR-2114*	MIMAT0011157	C-301490-00	CGAGCCUCAAGCAAGGGACUU	-1.02419077915881	0.00013793	TRUE	-2.57866858794879	0.057617	TRUE
hsa-miR-2117	MIMAT0011162	C-301496-00	UGUUCUCUUUGCCAAGGACAG	-0.806177347742896	0.00054279	FALSE	-2.55943646917884	0.057617	TRUE
hsa-miR-216a-3p	MIMAT0022844	C-301886-00	UCACAGUGGUCUCUGGGAUUU	-1.47806207137632	8.3715e-05	TRUE	-1.62257411616963	0.1633	FALSE
hsa-miR-22	MIMAT0000077	C-300493-03	AAGUCGCUUUCUAGAAACUGU	-1.14812626998085	0.00054279	FALSE	-2.8254651928315	0.009387	TRUE
hsa-miR-220b	MI0005529	C-301218-01	CCACCACCGUGUCGACACUU	-1.10856445415353	0.12653	FALSE	-2.50799687656707	0.11887	TRUE
hsa-miR-221*	MIMAT0004568	C-301163-01	ACCUGCAUACAAGUAGAUUU	-1.0647285020049	0.00016067	TRUE	0.493351763855846	0.86349	FALSE
hsa-miR-223*	MIMAT0004570	C-301197-01	CGUGUAUUUGACAAGCUGAGUU	-0.596047980424722	0.0074787	FALSE	-2.64001675119474	0.057617	TRUE
hsa-miR-2276	MIMAT0011775	C-301481-00	UAGUCGCUUUCUAGAGCCGAGG	-1.23563628890583	3.9917e-05	TRUE	-0.719842576977581	0.76975	FALSE
hsa-miR-2277-3p	MIMAT0011777	C-301482-00	UGACAGCGCCUUGCCUGGCUC	-1.22725149342576	3.9917e-05	TRUE	-2.2780178572373	0.11887	TRUE
hsa-miR-23a*	MIMAT0004496	C-301025-01	GGGGUUCUGGGGAUGGGAUUU	-0.773715976814457	0.0018747	FALSE	-2.22574058493535	0.11887	TRUE
hsa-miR-25*	MIMAT0004498	C-301183-01	AGGCGGAGACUUGGCAAUUG	-1.14410824968417	0.0018747	FALSE	-2.13159960818718	0.057617	TRUE
hsa-miR-2681-3p	MIMAT0013516	C-301978-00	UAUCAUGGAGUUGGUAAGCAC	-0.841542410878178	0.00023985	TRUE	-0.564927523090931	0.86349	FALSE
hsa-miR-2681-5p	MIMAT0013515	C-301977-00	GUUUUACCACUCCAGGAGACU	-1.20496402288836	3.6715e-05	TRUE	-2.40988448605506	0.057617	TRUE
hsa-miR-2682-3p	MIMAT0013518	C-301980-00	CGCCUCUUCAGCGCUGUCUUC	-0.908772237664288	0.00013793	TRUE	-1.43184476418853	0.29187	FALSE
hsa-miR-2682-5p	MIMAT0013517	C-301979-00	CAGGCAGUGACUGUUCAGACGUC	-1.24830345350196	1.5894e-05	TRUE	-2.80779774369284	0.014453	TRUE
hsa-miR-26b	MIMAT0000083	C-300501-07	UUCAAGUAUUUCAGGAUAGGU	-0.405072630482104	0.12653	FALSE	-2.50877510131769	0.081661	TRUE
hsa-miR-27a	MIMAT0000084	C-300502-03	UUCACAGUGGCUAAGUUCGCG	-1.32790971119818	3.9917e-05	TRUE	-1.95676458087438	0.1633	FALSE
hsa-miR-27a*	MIMAT0004501	C-301028-01	AGGGCUUAGCUGCUUGUGAGCA	-0.887009503478049	0.0052694	FALSE	-3.44308089121935	0.028781	TRUE
hsa-miR-27b*	MIMAT0004588	C-301154-01	AGAGCUUAGCUGAUUGGUGAUC	-1.00848877755825	0.0014577	FALSE	-2.34115081043794	0.050122	TRUE
hsa-miR-28-5p	MIMAT0000085	C-300503-05	AAGGAGUACAGCAGUUAUUGAG	-0.358344656239635	0.17522	FALSE	-1.5918060993224	0.11887	TRUE
hsa-miR-2861	MIMAT0013802	C-301642-00	GGGGCCUGGCGGUGGGCGG	-0.615484847088555	0.0046172	FALSE	-2.44784331747309	0.081661	TRUE
hsa-miR-2964a-3p	MIMAT0019748	C-302218-00	AGAAUUGCGUUUGGACAUCAGU	-1.13977573653624	6.1693e-05	TRUE	-0.558827938583115	0.76975	FALSE
hsa-miR-298	MIMAT0004901	C-301212-01	AGCAGAAGCAGGGAGGUUCUCCCA	-0.456122353506126	0.54004	FALSE	-2.83068032162142	0.11887	TRUE
hsa-miR-299-5p	MIMAT0002890	C-300854-03	UGUUUAACCGUCCCAUUAUUA	-1.25864926851518	0.00023985	TRUE	-0.317867194423722	1	FALSE
hsa-miR-299a*	MIMAT0004503	C-301178-01	ACUGAUUUUUUUGGUGUUCAG	-1.35357089379682	0.0021076	FALSE	-2.75349311494331	0.057617	TRUE
hsa-miR-29b-1*	MIMAT0004514	C-301150-01	GCUGUUUAUUAUGGUGUUUAGA	-0.970652939446982	0.015067	FALSE	-2.38342736203068	0.11887	TRUE
hsa-miR-301b	MIMAT0004958	C-301252-01	CAGUGCAUUAUUGUCAAAAGC	-0.837318501823261	0.0014577	FALSE	-2.28547978225747	0.11887	TRUE
hsa-miR-302b*	MIMAT0000714	C-300668-07	ACUUUAACUUGGAAGUGCUUUC	-0.550910910384927	0.037103	FALSE	-1.76045353694163	0.12226	TRUE
hsa-miR-302f	MIMAT0005932	C-301410-00	UAAUUGCUUCCAUGUUU	-1.5960005623826	1.5894e-05	TRUE	-3.17802223827817	0.014453	TRUE
hsa-miR-30b	MIMAT0000420	C-300590-03	UGUAAACAUCCUACACUCAGCU	-0.759206041294187	0.0018747	FALSE	-2.5817157170778	0.057617	TRUE
hsa-miR-30c-1*	MIMAT0004674	C-301199-01	CUGGGAGAGGGUUGUUUACUCC	-1.43787786865803	8.3715e-05	TRUE	0.291968781990601	1	FALSE
hsa-miR-30c-2*	MIMAT0004550	C-301034-01	CUGGGAGAAGGCGUUUUAUCUCU	-1.18964958597662	8.3715e-05	TRUE	-0.203715081689554	1	FALSE
hsa-miR-30d	MIMAT0000245	C-300543-03	UGUAAACAUCCCGACUGGAAG	-0.469794284958227	0.061384	FALSE	-2.24325814749711	0.08542	TRUE
hsa-miR-3115	MIMAT0014977	C-301644-00	AUAUGGGUUUACUAGUUGGU	-0.595634685516391	0.0052694	FALSE	-2.22531374934429	0.11887	TRUE
hsa-miR-3116	MIMAT0014978	C-301645-00	UGCCUGGAACAUAGUAGGGACU	-1.61818516940984	6.7884e-06	TRUE	-1.27627977004857	0.51048	FALSE
hsa-miR-3117	MIMAT0014979	C-301647-00	AUAGGACUCAUUAAGUCCAG	-1.07944692693698	6.1693e-05	TRUE	0.0583708702116637	1	FALSE
hsa-miR-3119	MIMAT0014981	C-301651-00	UGGCUUUUAACUUUGAUGGC	-0.77136494055742	0.00087976	FALSE	-2.20041353445194	0.11887	TRUE

Proviral effect

Note: Hits were selected using the following Local False Discovery Rate (lfr) thresholds: 0.00027 in screen part 1 and 0.1226 in screen part 2. Positive log₂ fold change values indicated a proviral effect of the miRNA on the HCV life cycle, while negative log₂ fold change values indicated an antiviral effect.

V. DISCUSSION

A genome-wide miRNA mimic screen in Huh7.5.1 cells to systematically uncover human miRNAs affecting the HCV replication cycle, especially HCV assembly and release of infectious virions, provided the basis of the present thesis. To this end, new achievements of virus-host interactions could be obtained: (i) miR-501-3p and miR-619-3p were found to enhance late steps of HCV infection (ii) using endogenous overexpression of miR-501-3p in human hepatoma cells, we have demonstrated that this miRNA specifically targets OGT and regulates its expression at the protein level, (iii) increased infectivity of HCV particles through silencing or inhibition of OGT's enzymatic activity, and (iv) release of bigger HCV particles after knock-down of OGT proposing that *O*-GlcNAcylation mediated by OGT affects HCV morphology. Whether miR-501-3p contributes to regulate the physiological expression of OGT in human hepatocytes remains elusive. Interestingly both miR-501-3p and *OGT* are located on the X chromosome. Overall, our data and data from the literature suggest an involvement of OGT and *O*-GlcNAcylation for HCV infection as well as for HCC progression, even if their role in each disease may be completely different.

Enveloped viruses consist of an host-derived envelope membrane with viral proteins more or less heavily glycosylated by the host glycosylation machinery as viruses pass through the secretory pathway protecting from the host immune system (e.g. HCV) or playing a crucial role for virus entry into host cells (e.g. Influenza virus) (Air 2014). While *N*-glycosylation of HCV E1 and E2 glycoproteins is well-defined as it serves as a protective glycan shield from the humoral immune response (reviewed in Helle, Duverlie et al. 2011), a link between *O*-GlcNAcylation and HCV infection had not been described before. *O*-GlcNAcylation is a crucial regulator for metabolic pathways within the liver (e.g. insulin signaling, bile acid metabolism and lipogenesis) (Yang and Qian 2017), however, due to the enormous number of *O*-GlcNAcyated substrates, detailed characterization of their role in HCV infection is lacking. Interestingly, proteins can either be *O*-GlcNAcyated or phosphorylated on the same residues resulting in adjusted cellular signaling (Hart, Slawson et al. 2011) (see p.28). Potential *O*-GlcNAcylation and phosphorylation at the same serine or threonine residue are defined as Yin Yang sites (Hart, Greis et al. 1995).

When we think retrospectively on the present results, since silencing of *OGT* through siRNAs or inhibition of its enzymatic activity by small molecules both lead to an enhancement of HCV infection as well as bigger particles proposing a potential role of *O*-GlcNAcylation for HCV morphology, one hypothesis could be that HCV envelope glycoproteins or LVP-associated host proteins are *O*-GlcNAcyated. However, since HCV assembly takes place in the ER lumen, and OGT/OGA are found in nucleocytoplasmic and mitochondrial compartments and so far, not known to localize to the ER lumen, a direct modulation of HCV viral proteins through *O*-GlcNAcylation seems unlikely, even if

E1 and E2 both contain putative *O*-GlcNAcylation sites (Bandiera and Zeisel, unpublished data and reviewed in Lavie, Hanouille et al. 2018).

Another important aspect could have been the potential *O*-GlcNAcylation of host-derived proteins associated with HCV, such as ApoE and ApoB. However, to our knowledge, although ApoE has been shown to be *O*-glycosylated, its *O*-GlcNAcylation by OGT has not been described. Indeed, ApoE which is primarily produced in the liver and associated with HDL and VLDL has been shown to be *O*-glycosylated at threonine residue 194 (Thr¹⁹⁴) (Wernette-Hammond, Lauer et al. 1989) and serine residue 290 (Ser²⁹⁰) (Lee, Kockx et al. 2010), while the Asn-X-Thr/Ser consensus sequence needed for *N*-glycosylation is lacking. Amino sugar analysis of monosialic apoE peptides revealed the presence of galactosamine, indicating the presence of GalNAc, but absence of glucosamine, indicating absence of GlcNAc (Wernette-Hammond, Lauer et al. 1989). In contrast to monosialic apoE, disialic apoE may exhibit a more complex carbohydrate structure as disialic apoE peptides contained both galactosamine and glucosamine (Wernette-Hammond, Lauer et al. 1989). It could be demonstrated that the *O*-glycosylation site Thr¹⁹⁴ is not important for secretion (Wernette-Hammond, Lauer et al. 1989) but Ser²⁹⁰ *O*-glycosylation that is situated at the C-terminus of ApoE, is important for ApoE stability, solubility and lipid binding (Lee, Kockx et al. 2010). In line with this, another study could reveal that long-term ethanol treatment decreased *O*-glycosylation of ApoE resulting in a shift of ApoE association from HDL to VLDL; indeed under alcoholic control decreased ApoE levels were found in association with VLDL (Ghosh, Liu et al. 1995). In contrast, ApoB molecules are highly *N*-glycosylated with 16 of 19 *N*-glycosylation sites found to be glycosylated (Fujioka, Taniguchi et al. 1994; Wong and Torbati 1994), while no *O*-glycosylation sites have been reported yet.

Another possibility than directly affecting the viral particle-associated proteins, could be the post-translational modulation of one or several HCV host factors required for HCV morphogenesis via OGT/OGA. *OGT*-silencing in the experimental settings used in the present paper showed no significant effect on early steps of HCV infection, even if some *O*-GlcNAcylation or Yin Yang sites of CLDN1 (Ahmad, Shabbiri et al. 2011; Butt, Khan et al. 2012) and OCLN (Butt, Feng et al. 2012) could be predicted suggesting that *O*-GlcNAcylation of CLDN1 and OCLN is not relevant for HCV infection. Ribosomal receptor or activated C-kinase 1 (RACK1), an adaptor protein and component of the 40S subunit of ribosomes, is a cellular factor required for IRES-dependent translation and replication of HCV in human hepatocytes without interacting with the miRNA pathway (Majzoub, Hafirassou et al. 2014). Interestingly, RACK1 is highly *O*-GlcNAcyated at Serine position 122 resulting in its stability and ribosome binding. Indeed, *O*-GlcNAcylation of RACK1 increases phosphorylation of eIF4E and translation of oncogenes in human hepatoma cells (Duan, Wu et al. 2018). Since increased *O*-GlcNAcylation is associated with tumorigenesis, angiogenesis and

metastasis, RACK1 has been reported as one host factor whose *O*-GlcNAcylation contributes to these processes in HCC patients and is correlated to tumor progression and recurrence after chemotherapy (Duan, Wu et al. 2018). Of note, Cao et al demonstrated that *O*-GlcNAcylation of eIF4E resulted in a stem-like cell potential of hepatoma cells contributing to HCC progression and tumorigenesis proposing two host *O*-GlcNAcylation host factors, eIF4E and RACK1, that built a bridge between *O*-GlcNAc metabolism and tumorigenesis (Duan, Wu et al. 2018; Cao, Duan et al. 2019).

Among the host factors involved in the HCV replication cycle, nuclear pore complexes (NPCs) consistent of nucleoporins (Nups) were also observed to be *O*-GlcNAcylation (Zhu, Liu et al. 2016). NPCs are hijacked by HCV and recruited to the membranous web during viral replication where they associate with viral proteins (core, NS5A, NS2 and NS3) contributing to the architecture of the membranous web and facilitating transport of viral and host proteins into the membranous web (Neufeldt, Joyce et al. 2013; Levin, Neufeldt et al. 2014). Depletion of Nup98, Nup153 and Nup155 appeared to inhibit HCV replication and assembly resulting in reduced intracellular levels of viral RNA and secreted virus, however, without affecting the specific infectivity of secreted virions (Neufeldt, Joyce et al. 2013). However, we could demonstrate here that knock-down of OGT resulted in an enhanced viral infection and an alteration of specific infectivity of viral particles leading to the conclusion that *O*-GlcNAcylation level of Nups does not contribute to the effects of OGT-silencing on HCVcc infectivity.

Besides HCV, *O*-GlcNAcylation and the action of OGT and OGA could be demonstrated to be involved in other viral infections: (i) Human immunodeficiency virus type 1 (HIV-1), Human T-cell leukemia virus type-1 (HTLV-1), Herpes simplex virus (HSV) and Kaposi's sarcoma-associated herpesvirus (KSHV) (Jochmann, Thureau et al. 2009; Jochmann, Pfannstiel et al. 2013; Groussaud, Khair et al. 2017; Angelova, Ortiz-Meoz et al. 2015). It can be assumed that the involvement of *O*-GlcNAcylation is as different as the viruses itself: (i) Overexpression of OGT as well as increased *O*-GlcNAcylation led to an repressed HIV-1 transcription as well as an decreased KSHV replication highlighting the link between viral replication and glucose metabolism (Jochmann, Thureau et al. 2009; Jochmann, Pfannstiel et al. 2013), (ii) Inhibition of OGA by HTLV-1 protein Tax led to an increased *O*-GlcNAcylation resulting in an increased viral transcription (Groussaud, Khair et al. 2017), and (iii) Inhibition of OGT resulted in a decreased HSV replication (Angelova, Ortiz-Meoz et al. 2015). Decreased OGT levels could be observed *in vitro* upon HCV infection (Herzog, Bandiera et al. 2019).

Of note, deregulation of *O*-GlcNAcylation is associated with a variety of cancers. Increased OGT expression as well as an enhanced *O*-GlcNAcylation could be observed in HCC tissue of HCV-infected patients. It was reported that OGT activates oncogenic signaling pathways in liver-derived cells via regulation of palmitic acid and the induction of ER stress thereby promoting tumor growth

and metastasis (Xu, Zhang et al. 2017), and additionally a link between *O*-GlcNAcylation and HCC recurrence after LT could be determined (Zhu, Zhou et al. 2012) proposing a potential role of *O*-GlcNAcylation and its enzymes, OGT and OGA, in the contribution to HCV-induced liver disease and hepatocarcinogenesis. As described in 2.3, an increased flux through glycolysis is observed in cancer cells, additionally, it has been described that cancer cells show altered lipogenesis pathways supporting membrane synthesis and generating signaling molecules resulting in rapid cell growth (Baenke, Peck et al. 2013); indeed, cancer cells utilize *de novo* lipogenesis instead of obtaining lipids from the bloodstream (Swinnen, Brusselmans et al. 2006) involving activation and expression of enzymes able to generate lipids such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), ATP-citrate lyase (ACLY) as well as the transcription factor that regulates these enzymes, termed sterol regulatory element binding protein (SREBP-1) (Santos and Schulze 2012). Recently could be demonstrated that *O*-GlcNAcylation is a crucial factor in directly regulating SREBP-1 phosphorylation and stability as well as controlling ACLY and FAS (Baldini, Wavelet et al. 2016) resulting in the regulation of lipid metabolism and cancer cell growth and survival (Sodi, Bacigalupa et al. 2018). Moreover, liver X receptor (LXR), another key transcriptional factor to regulate *de novo* lipogenesis, has been shown to be *O*-GlcNAcylated in response to glucose and to directly transactivate SREBP-1 (Bindesboll, Fan et al. 2015). These results highlight that in addition to its role in *O*-GlcNAcylation of oncogenic proteins, OGT is directly linked to the lipid metabolism in (cancer) cells and might serve as a therapeutic approach against highly metabolic cancer.

Of note, in addition to HCV, another oncogenic virus, Human papillomavirus 16 (HPV16), has been linked to OGT as well. Interestingly, it could be demonstrated that levels of HVP16 oncoproteins E6 and E7 are modulated through OGT expression (Kim, Kim et al. 2016), while in turn upregulation of OGT through E6 resulted in an increase of *O*-GlcNAcylation and an activation of oncogenic activity of HPV (Zeng, Zhao et al. 2016) proposing a general involvement of OGT and *O*-GlcNAcylation in virus-induced cancer.

Apart from *O*-GlcNAcylation and to close the circle of the present work, miR-501-3p who can functionally target OGT (Herzog, Bandiera et al. 2019), has been shown to be involved in the formation of metastasis in HCC (Luo, Yin et al. 2018). Indeed, it could be demonstrated that a downregulation of miR-501-3p is associated with tumor progression and poor prognosis in patients with HCC, while in contrast overexpression of this miRNA could inhibit cancer cell proliferation, migration and evasion (Luo, Yin et al. 2018). This would be in line with the finding that *O*-GlcNAcylation is increased in HCC and possibly contribute to hepatocarcinogenesis.

VI. RÉSUMÉ DE LA THÈSE DE DOCTORAT

Etude du rôle de l'OGT dans les étapes tardives du cycle de réplication du virus de l'hépatite C

6.1 Introduction

L'infection chronique par le virus de l'hépatite C (HCV) est un facteur de risque majeur de maladies hépatiques et de carcinome hépatocellulaire (CHC), la seconde cause de mortalité par cancer dans le monde. Depuis la mise sur le marché d'antiviraux à action directe qui ciblent tous les génotypes du HCV, l'hépatite C chronique peut être guérie chez plus de 90% des patients traités (Baumert, Juhling et al. 2017). Cependant, il est estimé que 71 millions d'individus sont toujours infectés par le HCV dans le monde et la guérison virale n'élimine pas le risque de développer un CHC lorsque la maladie hépatique est avancée (Baumert, Juhling et al. 2017; Hamdane, Juhling et al. 2019). Les traitements curatifs du CHC sont limités à la résection chirurgicale et la transplantation hépatique. De plus, pour prévenir la transmission globale de l'infection par le HCV, il sera primordial de développer un vaccin préventif.

L'infection des hépatocytes humains par le HCV est un processus complexe qui fait intervenir des facteurs viraux et cellulaires. L'identification de facteurs de l'hôte détournés par le HCV contribue à une meilleure compréhension des interactions virus-hôte sous-tendant le cycle de réplication du HCV et le développement d'une infection chronique ainsi qu'à l'identification de cibles potentielles pour le traitement des maladies hépatiques et la prévention du CHC (Zeisel and Baumert 2017; Zeisel, Crouchet et al. 2015). Le cycle de réplication du HCV peut être divisé en étapes précoces (entrée, traduction et réplication virale) et tardives (assemblage et relargage de nouveaux virions). Chacune de ces étapes dépend d'interactions virus-hôte qui mettent en jeu des protéines et microARN (miR) de l'hôte (Zeisel, Crouchet et al. 2015). Ces derniers constituent une classe de petits ARN non-codants qui régulent l'expression de gènes au niveau post-transcriptionnel. Ainsi ils contrôlent l'expression d'environ 60% de gènes codant des protéines et sont impliqués dans tous les processus biologiques. Les miR ciblent spécifiquement des ARN messagers (ARNm) par appariement de bases avec un site complémentaire qui est habituellement localisé dans la région 3' non-traduite (3'UTR). Ceci conduit généralement à la dégradation de l'ARNm ou à la répression de sa traduction en protéine. De récentes études indiquent que les miR contribuent à la réplication du HCV en exerçant des effets pro- ou antiviraux. La découverte du ciblage direct du HCV par miR-122, un miR fortement exprimé dans le foie, a permis de mettre en lumière son rôle essentiel dans la traduction et la réplication du HCV en stabilisant l'ARN HCV (Jopling, Yi et al. 2005). miR-122 est un facteur proviral qui joue un rôle

dans l'infection chronique par le HCV, la progression de la maladie hépatique et le CHC (Jopling, Yi et al. 2005). D'autres miR ont un effet indirect sur le HCV en modulant l'expression de protéines de l'hôte qui régulent les réponses antivirales et la surveillance immunitaire (Li, Lowey et al. 2017; Bandiera, Pfeffer et al. 2015; Li, Jiang et al. 2016). Par leur capacité à réguler l'expression génique, les miR représentent des outils pour des études de perte de fonction visant à découvrir de nouveaux facteurs de l'hôte impliqués dans le cycle de réplication du HCV.

Afin d'identifier de manière systématique les miR impliqués dans le cycle de réplication du HCV, le laboratoire a précédemment conduit un criblage dans les cellules hépatocytaires Huh7.5.1 en utilisant une librairie génomique de miR et un virus portant un gène rapporteur luciférase (JcR2a). Ce criblage a permis d'identifier 495 miR qui modulent significativement l'infection par le HCV. Parmi ces miR, 186 miR sont impliqués dans les étapes précoces du cycle viral et 309 miR jouent un rôle dans l'assemblage et le relargage, deux étapes encore peu caractérisées du cycle viral. Le laboratoire a ainsi décidé d'étudier plus particulièrement les miR qui modulent les étapes tardives du cycle de réplication du HCV. Parmi ces miR, miR-501-3p et miR-619-3p augmentent l'infection par le HCV, ce qui suggère qu'ils régulent des facteurs de l'hôte qui jouent un rôle dans la morphogénèse ou la sécrétion de virions. Par une approche combinant outils bioinformatiques et études fonctionnelles, le laboratoire a identifié l'OGT (*O-linked N-acetylglucosamine transferase* ou UDP-N-acétylglucosamine-peptide N-acétylglucosaminyltransférase ou O-GlcNAc transférase) comme cible de miR-501-3p et miR-619-3p impliquée dans l'assemblage et l'infectivité du HCV (Herzog, Bandiera et al. 2019). L'OGT est une enzyme qui catalyse l'addition de N-acétylglucosamine (O-GlcNAc) à des résidus sérine et thréonine de protéines nucléaires, cytoplasmiques et mitochondriales (O-GlcNAcylation) (Levine and Walker 2016). En plus de sa fonction enzymatique, l'OGT contribue à la stabilisation de protéines dans des complexes multiprotéines (fonction d'échaffaudage ou *scaffold*). Cette modification post-traductionnelle est hydrolysée par l'OGA (N-acétyl-b-D glucosaminidase ou O-GlcNAcase). L'OGT et l'OGA sont les deux seules enzymes responsables de la modulation de l'O-GlcNAcylation des protéines (Levine and Walker 2016).

6.2 Objectifs

Le but de ma thèse a été d'étudier le rôle de l'OGT dans les interactions HCV-hôte. Les objectifs spécifiques étaient i) de caractériser le ciblage prédit de l'OGT par miR-501-3p et miR-619-3p, ii) de déterminer comment l'OGT pouvait contribuer à la morphogénèse du HCV et iii) d'étudier le rôle de miR-501-3p et de l'OGT dans la maladie hépatique induite par HCV et le CHC.

6.3.1 miR-501-3p régule l'expression de l'OGT au niveau post-transcriptionnel

Pour caractériser la régulation de l'OGT par miR-501-3p et miR-619-3p, l'expression de l'OGT a été analysée au niveau de l'ARNm et au niveau protéique dans les cellules Huh7.5.1 après surexpression de miR-501-3p et miR-619-3p. Tandis qu'aucun miR n'avait d'impact sur les niveaux d'ARNm de l'OGT, la surexpression de miR-501-3p a significativement diminué l'expression de la protéine OGT. miR-619-3p a également diminué l'expression protéique de l'OGT mais de manière moins robuste que miR-501-3p. Ainsi nous avons concentré nos travaux sur miR-501-3p.

Pour déterminer si l'OGT est une cible de miR-501-3p, un fragment de la région 3'UTR de l'ARNm de l'OGT contenant un site potentiel de fixation pour miR-501-3p a été sous-cloné dans une cassette d'expression de luciférase *Renilla* (RLuc) d'un vecteur double rapporteur exprimant également la luciférase *Firefly*. La co-transfection de miR-501-3p avec le vecteur rapporteur contenant la séquence 3'UTR sauvage (RLuc wt *OGT* 3'UTR) a significativement diminué l'activité luciférase comparé à la co-transfection avec le vecteur vide. Par contre, cette répression de l'activité luciférase n'a pas été observée lorsqu'un vecteur contenant un site de fixation pour miR-501-3p muté a été co-transfecté (RLuc mt *OGT* 3'UTR). L'ensemble de ces résultats indique que miR-501-3p régule l'expression post-transcriptionnelle de l'OGT en diminuant son expression protéique.

6.3.2 La O-GlcNAcylation module l'infectivité des HCVcc

Pour déterminer l'effet de l'OGT sur l'assemblage et le relargage du HCV, nous avons utilisé de petits ARN interférants (siARN) pour diminuer l'expression de l'OGT dans les cellules Huh7.5.1. Les siARN sont des molécules ARN double brin d'environ 20-25 paires de bases qui, comme les miR, régulent l'expression de gènes en dégradant spécifiquement certains ARNm pour empêcher leur traduction. Nous avons ensuite déterminé les titres infectieux HCV (TCID₅₀) et les taux d'ARN HCV pour calculer l'infectivité spécifique des particules HCVcc produites dans les cellules Huh7.5.1 dans lesquelles l'expression de l'OGT a été diminuée. De manière intéressante, la diminution de l'expression de l'OGT a conduit à une diminution significative des TCID₅₀ et l'infectivité spécifique des HCVcc. Cet effet de l'OGT sur l'infectivité des HCVcc n'est pas dépendant du génotype HCV étant donné qu'une augmentation de l'infectivité des HCVcc comportant les glycoprotéines d'enveloppe des génotypes 1a, 1b et 2a a été observée dans les conditions où l'expression de l'OGT a été diminuée. Afin de déterminer si l'activité enzymatique de l'OGT module l'infectivité des HCVcc, nous avons incubé des cellules Huh7.5.1 infectées par des HCVcc avec deux inhibiteurs pharmacologiques ciblant l'OGT (Ac₄5S-GlcNAc) ou l'OGA (Thiamet G). Le traitement par l'Ac₄5S-

GlcNAc a augmenté significativement l'infectivité des HCVcc de manière dose-dépendante, tandis que l'effet opposé a été observé après le traitement par le Thiamet G. L'ensemble de ces résultats montre que la O-GlcNAcylation module l'infectivité des HCVcc.

6.3.3 La diminution de l'expression de l'OGT module les propriétés biophysiques et la taille des HCVcc

Au cours de la morphogénèse virale, les glycoprotéines d'enveloppe du HCV interagissent étroitement avec les apolipoprotéines B et E (ApoB et ApoE) de l'hôte, ce qui contribue à masquer les épitopes viraux à la surface des lipo-viro-particules (LVPs) (Bartenschlager, Penin et al. 2011). Afin de déterminer l'effet de l'OGT sur la morphogénèse virale, nous avons analysé les propriétés structurales et biophysiques des HCVcc produits dans des cellules Huh7.5.1 transfectées avec des siOGT ou des siARN contrôle après ultracentrifugation en gradients d'iodixanol. La diminution de l'expression de l'OGT a conduit à une production d'HCVcc plus infectieux, d'une densité plus élevée et avec des concentrations supérieures en ApoE. Ces résultats indiquent que l'OGT a un impact sur les propriétés biophysiques des HCVcc. A l'inverse, aucun changement dans les concentrations en ApoB n'a été observé, en accord avec le modèle selon lequel les HCV LVPs contiendraient plusieurs molécules échangeables d'ApoE mais une seule molécule d'ApoB. Nous avons également purifié les HCVcc pour les visualiser par microscopie électronique après immunocapture par un anticorps dirigé contre la protéine d'enveloppe E2 du HCV (Piver, Boyer et al. 2017) pour déterminer si la diminution de l'expression de l'OGT avait un impact sur la taille des HCVcc. En comparaison avec les particules virales produites dans les conditions contrôles, les particules virales produites dans des cellules Huh7.5.1 qui avaient été préalablement transfectées avec les siOGT présentaient des tailles plus grandes. Il est intéressant de noter que des particules plus grandes ont été observées dans plusieurs fractions du gradient d'iodixanol dans lesquels les HCVcc présentaient une infectivité et une concentration en ApoE plus élevées. Ces résultats suggèrent que la diminution de l'expression de l'OGT a un impact sur la lipitation des HCVcc.

6.3.4 L'expression de l'OGT augmente dans les maladies hépatiques

Etant donné que la diminution de l'expression de l'OGT conduit à la production d'HCVcc plus infectieuses, nous nous sommes interrogés si l'infection par le HCV pouvait avoir un effet sur l'expression de l'OGT afin de promouvoir le cycle viral. Dans les cellules Huh7.5.1, l'infection par le HCV a conduit à une diminution de l'expression de l'ARNm et des protéines OGT qui pourraient ainsi augmenter l'infection par le HCV, en accord avec le rôle antiviral de la O-GlcNAcylation que nous avons observé. Cependant, dans les tissus hépatiques de patients atteints d'hépatite C chronique, les taux d'ARN HCV n'étaient pas corrélés avec l'expression de l'OGT, suggérant que chez les patients il n'y a pas d'effet direct du HCV sur l'expression de l'OGT. Ensuite, étant donné que la O-

GlcNAcylation a été associée à de nombreux cancers, nous avons également analysé l'expression de l'OGT dans des tissus hépatiques de patients atteints de maladies hépatiques chroniques et de CHC. Alors que nous n'avons observé qu'une tendance à l'augmentation de l'expression de l'OGT dans des tissus hépatiques de patients atteints d'hépatite C et présentant une fibrose et une inflammation hépatique, les taux d'expression de l'OGT étaient significativement augmentés dans les tissus tumoraux de patients chroniquement infectés par le HCV ou par le virus de l'hépatite B ainsi que de patients atteints de maladie hépatique alcoolique ou non-alcoolique en comparaison avec des tissus hépatiques non tumoraux. Ces résultats suggèrent que l'expression de l'OGT augmente dans le CHC de manière indépendante de l'étiologie du cancer. L'ensemble de ces résultats suggère que l'augmentation de l'expression de l'OGT est liée à l'inflammation et à la fibrose hépatique au cours de la maladie viro-induite plutôt qu'à un effet direct du virus..

6.4 Conclusions et perspectives

Au cours de ma thèse, nous avons découvert un rôle pour l'OGT dans l'infection par le HCV. Nous avons montré que i) miR-501-3p régule l'expression de l'OGT au niveau protéique; ii) la diminution de l'expression de l'OGT par des siARN ou l'inhibition de son activité enzymatique augmente l'infectivité des particules virales; iii) la diminution de l'expression de l'OGT conduit à la production de particules virales présentant des tailles plus grandes suggérant que la O-GlcNAcylation a un impact sur la morphogénèse et l'infectivité du HCV; et iv) l'expression de l'OGT augmente au cours des maladies hépatiques chroniques et du CHC.

En conclusion, en identifiant l'OGT comme nouveau facteur de l'hôte régulant la morphogénèse du HCV, notre étude permet de mieux comprendre les interactions HCV-hôte modulant l'infection virale. Etant donné qu'il a été précédemment montré que l'OGT jouait un rôle dans les modifications métaboliques au cours du cancer (Jopling, Yi et al. 2005), nos résultats suggèrent que l'OGT pourrait contribuer au développement de la maladie hépatique induite par le HCV et du CHC.

VII. REFERENCES

- Abe, K., T. Kurata, Y. Teramoto, J. Shiga, and T. Shikata. 1993. 'Lack of susceptibility of various primates and woodchucks to hepatitis C virus', *J Med Primatol*, 22: 433-4.
- Afdhal, N., K. R. Reddy, D. R. Nelson, E. Lawitz, S. C. Gordon, E. Schiff, R. Nahass, R. Ghalib, N. Gitlin, R. Herring, J. Lalezari, Z. H. Younes, P. J. Pockros, A. M. Di Bisceglie, S. Arora, G. M. Subramanian, Y. Zhu, H. Dvory-Sobol, J. C. Yang, P. S. Pang, W. T. Symonds, J. G. McHutchison, A. J. Muir, M. Sulkowski, and P. Kwo. 2014. 'Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection', *N Engl J Med*, 370: 1483-93.
- Afdhal, N., S. Zeuzem, P. Kwo, M. Chojkier, N. Gitlin, M. Puoti, M. Romero-Gomez, J. P. Zarski, K. Agarwal, P. Buggisch, G. R. Foster, N. Brau, M. Buti, I. M. Jacobson, G. M. Subramanian, X. Ding, H. Mo, J. C. Yang, P. S. Pang, W. T. Symonds, J. G. McHutchison, A. J. Muir, A. Mangia, and P. Marcellin. 2014. 'Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection', *N Engl J Med*, 370: 1889-98.
- Ahmad, W., K. Shabbiri, B. Ijaz, S. Asad, M. T. Sarwar, S. Gull, H. Kausar, K. Fouzia, I. Shahid, and S. Hassan. 2011. 'Claudin-1 required for HCV virus entry has high potential for phosphorylation and O-glycosylation', *Virol J*, 8: 229.
- Air, G. M. 2014. 'Influenza virus-glycan interactions', *Curr Opin Virol*, 7: 128-33.
- Aizaki, H., K. J. Lee, V. M. Sung, H. Ishiko, and M. M. Lai. 2004. 'Characterization of the hepatitis C virus RNA replication complex associated with lipid rafts', *Virology*, 324: 450-61.
- Aizaki, H., K. Morikawa, M. Fukasawa, H. Hara, Y. Inoue, H. Tani, K. Saito, M. Nishijima, K. Hanada, Y. Matsuura, M. M. Lai, T. Miyamura, T. Wakita, and T. Suzuki. 2008. 'Critical role of virion-associated cholesterol and sphingolipid in hepatitis C virus infection', *J Virol*, 82: 5715-24.
- Albecka, A., R. Montserret, T. Krey, A. W. Tarr, E. Diesis, J. K. Ball, V. Descamps, G. Duverlie, F. Rey, F. Penin, and J. Dubuisson. 2011. 'Identification of new functional regions in hepatitis C virus envelope glycoprotein E2', *J Virol*, 85: 1777-92.
- Alisson-Silva, F., L. Freire-de-Lima, J. L. Donadio, M. C. Lucena, L. Penha, J. N. Sa-Diniz, W. B. Dias, and A. R. Todeschini. 2013. 'Increase of O-glycosylated oncofetal fibronectin in high glucose-induced epithelial-mesenchymal transition of cultured human epithelial cells', *PLoS One*, 8: e60471.
- Amako, Y., K. Tsukiyama-Kohara, A. Katsume, Y. Hirata, S. Sekiguchi, Y. Tobita, Y. Hayashi, T. Hishima, N. Funata, H. Yonekawa, and M. Kohara. 2010. 'Pathogenesis of hepatitis C virus infection in *Tupaia belangeri*', *J Virol*, 84: 303-11.
- Andre, P., F. Komurian-Pradel, S. Deforges, M. Perret, J. L. Berland, M. Sodoyer, S. Pol, C. Brechot, G. Paranhos-Baccala, and V. Lotteau. 2002. 'Characterization of low- and very-low-density hepatitis C virus RNA-containing particles', *J Virol*, 76: 6919-28.
- Andre, P., G. Perlemuter, A. Budkowska, C. Brechot, and V. Lotteau. 2005. 'Hepatitis C virus particles and lipoprotein metabolism', *Semin Liver Dis*, 25: 93-104.
- Angelova, M., R. F. Ortiz-Meoz, S. Walker, and D. M. Knipe. 2015. 'Inhibition of O-Linked N-Acetylglucosamine Transferase Reduces Replication of Herpes Simplex Virus and Human Cytomegalovirus', *J Virol*, 89: 8474-83.
- Antaki, N., A. Craxi, S. Kamal, R. Moucari, S. Van der Merwe, S. Haffar, A. Gadano, N. Zein, C. L. Lai, J. M. Pawlotsky, E. J. Heathcote, G. Dusheiko, and P. Marcellin. 2010. 'The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report', *Liver Int*, 30: 342-55.
- Appel, N., T. Pietschmann, and R. Bartenschlager. 2005. 'Mutational analysis of hepatitis C virus nonstructural protein 5A: potential role of differential phosphorylation in RNA replication and identification of a genetically flexible domain', *J Virol*, 79: 3187-94.

- Asselah, T., N. Boyer, D. Saadoun, M. Martinot-Peignoux, and P. Marcellin. 2016. 'Direct-acting antivirals for the treatment of hepatitis C virus infection: optimizing current IFN-free treatment and future perspectives', *Liver Int*, 36 Suppl 1: 47-57.
- Azuma, H., N. Paulk, A. Ranade, C. Dorrell, M. Al-Dhalimy, E. Ellis, S. Strom, M. A. Kay, M. Finegold, and M. Grompe. 2007. 'Robust expansion of human hepatocytes in Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-} mice', *Nat Biotechnol*, 25: 903-10.
- Bacon, B. R., S. C. Gordon, E. Lawitz, P. Marcellin, J. M. Vierling, S. Zeuzem, F. Poordad, Z. D. Goodman, H. L. Sings, N. Boparai, M. Burroughs, C. A. Brass, J. K. Albrecht, and R. Esteban. 2011. 'Boceprevir for previously treated chronic HCV genotype 1 infection', *N Engl J Med*, 364: 1207-17.
- Baenke, F., B. Peck, H. Miess, and A. Schulze. 2013. 'Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development', *Dis Model Mech*, 6: 1353-63.
- Bailey, J. R., E. Barnes, and A. L. Cox. 2019. 'Approaches, Progress, and Challenges to Hepatitis C Vaccine Development', *Gastroenterology*, 156: 418-30.
- Baktash, Y., A. Madhav, K. E. Collier, and G. Randall. 2018. 'Single Particle Imaging of Polarized Hepatoma Organoids upon Hepatitis C Virus Infection Reveals an Ordered and Sequential Entry Process', *Cell Host Microbe*, 23: 382-94 e5.
- Baldini, S. F., C. Wavelet, I. Hainault, C. Guinez, and T. Lefebvre. 2016. 'The Nutrient-Dependent O-GlcNAc Modification Controls the Expression of Liver Fatty Acid Synthase', *J Mol Biol*, 428: 3295-304.
- Ball, J. K., A. W. Tarr, and J. A. McKeating. 2014. 'The past, present and future of neutralizing antibodies for hepatitis C virus', *Antiviral Res*, 105: 100-11.
- Bandiera, S., S. Pfeffer, T. F. Baumert, and M. B. Zeisel. 2015. 'miR-122--a key factor and therapeutic target in liver disease', *J Hepatol*, 62: 448-57.
- Banerjee, P. S., O. Lagerlof, and G. W. Hart. 2016. 'Roles of O-GlcNAc in chronic diseases of aging', *Mol Aspects Med*, 51: 1-15.
- Barkovskaya, A., K. Seip, B. Hilmarisdottir, G. M. Maelandsmo, S. A. Moestue, and H. M. Ikonen. 2019. 'O-GlcNAc Transferase Inhibition Differentially Affects Breast Cancer Subtypes', *Sci Rep*, 9: 5670.
- Barouch-Bentov, R., G. Neveu, F. Xiao, M. Beer, E. Bekerman, S. Schor, J. Campbell, J. Boonyaratanakornkit, B. Lindenbach, A. Lu, Y. Jacob, and S. Einav. 2016. 'Hepatitis C Virus Proteins Interact with the Endosomal Sorting Complex Required for Transport (ESCRT) Machinery via Ubiquitination To Facilitate Viral Envelopment', *MBio*, 7.
- Bartenschlager, R., T. F. Baumert, J. Bukh, M. Houghton, S. M. Lemon, B. D. Lindenbach, V. Lohmann, D. Moradpour, T. Pietschmann, C. M. Rice, R. Thimme, and T. Wakita. 2018. 'Critical challenges and emerging opportunities in hepatitis C virus research in an era of potent antiviral therapy: Considerations for scientists and funding agencies', *Virus Res*, 248: 53-62.
- Bartenschlager, R., and V. Lohmann. 2000. 'Replication of hepatitis C virus', *J Gen Virol*, 81: 1631-48.
- Bartenschlager, R., V. Lohmann, T. Wilkinson, and J. O. Koch. 1995. 'Complex formation between the NS3 serine-type proteinase of the hepatitis C virus and NS4A and its importance for polyprotein maturation', *J Virol*, 69: 7519-28.
- Bartenschlager, R., F. Penin, V. Lohmann, and P. Andre. 2011. 'Assembly of infectious hepatitis C virus particles', *Trends Microbiol*, 19: 95-103.
- Bartosch, B., J. Dubuisson, and F. L. Cosset. 2003. 'Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes', *J Exp Med*, 197: 633-42.
- Bartosch, B., A. Vitelli, C. Granier, C. Goujon, J. Dubuisson, S. Pascale, E. Scarselli, R. Cortese, A. Nicosia, and F. L. Cosset. 2003. 'Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor', *J Biol Chem*, 278: 41624-30.

- Bassett, S. E., K. M. Brasky, and R. E. Lanford. 1998. 'Analysis of hepatitis C virus-inoculated chimpanzees reveals unexpected clinical profiles', *J Virol*, 72: 2589-99.
- Baumert, T. F., F. Juhling, A. Ono, and Y. Hoshida. 2017. 'Hepatitis C-related hepatocellular carcinoma in the era of new generation antivirals', *BMC Med*, 15: 52.
- Bayer, K., C. Banning, V. Bruss, L. Wiltzer-Bach, and M. Schindler. 2016. 'Hepatitis C Virus Is Released via a Noncanonical Secretory Route', *J Virol*, 90: 10558-73.
- Behrens, S. E., L. Tomei, and R. De Francesco. 1996. 'Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus', *EMBO J*, 15: 12-22.
- Benga, W. J., S. E. Krieger, M. Dimitrova, M. B. Zeisel, M. Parnot, J. Lupberger, E. Hildt, G. Luo, J. McLauchlan, T. F. Baumert, and C. Schuster. 2010. 'Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles', *Hepatology*, 51: 43-53.
- Bindesboll, C., Q. Fan, R. C. Norgaard, L. MacPherson, H. B. Ruan, J. Wu, T. A. Pedersen, K. R. Steffensen, X. Yang, J. Matthews, S. Mandrup, H. I. Nebb, and L. M. Gronning-Wang. 2015. 'Liver X receptor regulates hepatic nuclear O-GlcNAc signaling and carbohydrate responsive element-binding protein activity', *J Lipid Res*, 56: 771-85.
- Bissig, K. D., T. T. Le, N. B. Woods, and I. M. Verma. 2007. 'Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model', *Proc Natl Acad Sci U S A*, 104: 20507-11.
- Biwi, J., C. Clarisse, C. Biot, R. P. Kozak, K. Madunic, M. Mortuaire, M. Wuhler, D. I. R. Spencer, C. Schulz, Y. Guerardel, T. Lefebvre, and A. S. Vercoutter-Edouart. 2019. 'OGT Controls the Expression and the Glycosylation of E-cadherin, and Affects Glycosphingolipid Structures in Human Colon Cell Lines', *Proteomics*: e1800452.
- Blanchard, E., S. Belouzard, L. Goueslain, T. Wakita, J. Dubuisson, C. Wychowski, and Y. Rouille. 2006. 'Hepatitis C virus entry depends on clathrin-mediated endocytosis', *J Virol*, 80: 6964-72.
- Blight, K. J., J. A. McKeating, and C. M. Rice. 2002. 'Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication', *J Virol*, 76: 13001-14.
- Bond, M. R., and J. A. Hanover. 2015. 'A little sugar goes a long way: the cell biology of O-GlcNAc', *J Cell Biol*, 208: 869-80.
- Boulant, S., M. W. Douglas, L. Moody, A. Budkowska, P. Targett-Adams, and J. McLauchlan. 2008. 'Hepatitis C virus core protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner', *Traffic*, 9: 1268-82.
- Boulant, S., R. Montserret, R. G. Hope, M. Ratinier, P. Targett-Adams, J. P. Lavergne, F. Penin, and J. McLauchlan. 2006. 'Structural determinants that target the hepatitis C virus core protein to lipid droplets', *J Biol Chem*, 281: 22236-47.
- Bourliere, M., O. Pietri, P. Castellani, V. Oules, and X. Adhoute. 2018. 'Sofosbuvir, velpatasvir and voxilaprevir: a new triple combination for hepatitis C virus treatment. One pill fits all? Is it the end of the road?', *Therap Adv Gastroenterol*, 11: 1756284818812358.
- Boyer, A., J. Dreneau, A. Dumans, J. Burlaud-Gaillard, A. Bull-Maurer, P. Roingeard, and J. C. Meunier. 2019. 'Endoplasmic Reticulum Detergent-Resistant Membranes Accommodate Hepatitis C Virus Proteins for Viral Assembly', *Cells*, 8.
- Bradley, D., K. McCaustland, K. Krawczynski, J. Spelbring, C. Humphrey, and E. H. Cook. 1991. 'Hepatitis C virus: buoyant density of the factor VIII-derived isolate in sucrose', *J Med Virol*, 34: 206-8.
- Brimacombe, C. L., J. Grove, L. W. Meredith, K. Hu, A. J. Syder, M. V. Flores, J. M. Timpe, S. E. Krieger, T. F. Baumert, T. L. Tellinghuisen, F. Wong-Staal, P. Balfe, and J. A. McKeating. 2011. 'Neutralizing antibody-resistant hepatitis C virus cell-to-cell transmission', *J Virol*, 85: 596-605.
- Buse, M. G. 2006. 'Hexosamines, insulin resistance, and the complications of diabetes: current status', *Am J Physiol Endocrinol Metab*, 290: E1-e8.

- Butkinaree, C., K. Park, and G. W. Hart. 2010. 'O-linked beta-N-acetylglucosamine (O-GlcNAc): Extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress', *Biochim Biophys Acta*, 1800: 96-106.
- Butt, A. M., D. Feng, I. Nasrullah, S. Tahir, M. Idrees, Y. Tong, and J. Lu. 2012. 'Computational identification of interplay between phosphorylation and O-beta-glycosylation of human occludin as potential mechanism to impair hepatitis C virus entry', *Infect Genet Evol*, 12: 1235-45.
- Butt, A. M., I. B. Khan, M. Hussain, M. Idress, J. Lu, and Y. Tong. 2012. 'Role of post translational modifications and novel crosstalk between phosphorylation and O-beta-GlcNAc modifications in human claudin-1, -3 and -4', *Mol Biol Rep*, 39: 1359-69.
- Caldwell, S. A., S. R. Jackson, K. S. Shahriari, T. P. Lynch, G. Sethi, S. Walker, K. Vosseller, and M. J. Reginato. 2010. 'Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1', *Oncogene*, 29: 2831-42.
- Camus, G., E. Herker, A. A. Modi, J. T. Haas, H. R. Ramage, R. V. Farese, Jr., and M. Ott. 2013. 'Diacylglycerol acyltransferase-1 localizes hepatitis C virus NS5A protein to lipid droplets and enhances NS5A interaction with the viral capsid core', *J Biol Chem*, 288: 9915-23.
- Cao, B., M. Duan, Y. Xing, C. Liu, F. Yang, Y. Li, T. Yang, Y. Wei, Q. Gao, and J. Jiang. 2019. 'O-GlcNAc transferase activates stem-like cell potential in hepatocarcinoma through O-GlcNAcylation of eukaryotic initiation factor 4E', *J Cell Mol Med*, 23: 2384-98.
- Carithers, R. L., Jr., and S. S. Emerson. 1997. 'Therapy of hepatitis C: meta-analysis of interferon alfa-2b trials', *Hepatology*, 26: 83s-88s.
- Catanese, M. T., K. Uryu, M. Kopp, T. J. Edwards, L. Andrus, W. J. Rice, M. Silvestry, R. J. Kuhn, and C. M. Rice. 2013. 'Ultrastructural analysis of hepatitis C virus particles', *Proc Natl Acad Sci U S A*, 110: 9505-10.
- Chang, K. S., J. Jiang, Z. Cai, and G. Luo. 2007. 'Human apolipoprotein e is required for infectivity and production of hepatitis C virus in cell culture', *J Virol*, 81: 13783-93.
- Cheng, Y. U., H. Li, J. Li, J. Li, Y. Gao, and B. Liu. 2016. 'O-GlcNAcylation enhances anaplastic thyroid carcinoma malignancy', *Oncol Lett*, 12: 572-78.
- Chong, W. M., S. C. Hsu, W. T. Kao, C. W. Lo, K. Y. Lee, J. S. Shao, Y. H. Chen, J. Chang, S. S. Chen, and M. J. Yu. 2016. 'Phosphoproteomics Identified an NS5A Phosphorylation Site Involved in Hepatitis C Virus Replication', *J Biol Chem*, 291: 3918-31.
- Choo, Q. L., G. Kuo, A. J. Weiner, L. R. Overby, D. W. Bradley, and M. Houghton. 1989. 'Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome', *Science*, 244: 359-62.
- Chung, R. T., and T. F. Baumert. 2014. 'Curing chronic hepatitis C--the arc of a medical triumph', *N Engl J Med*, 370: 1576-8.
- Cocquerel, L., A. Op de Beeck, M. Lambot, J. Roussel, D. Delgrange, A. Pillez, C. Wychowski, F. Penin, and J. Dubuisson. 2002. 'Topological changes in the transmembrane domains of hepatitis C virus envelope glycoproteins', *EMBO J*, 21: 2893-902.
- Colpitts, C. C., R. G. Tawar, L. Mailly, C. Thumann, L. Heydmann, S. C. Durand, F. Xiao, E. Robinet, P. Pessaux, M. B. Zeisel, and T. F. Baumert. 2018. 'Humanisation of a claudin-1-specific monoclonal antibody for clinical prevention and cure of HCV infection without escape', *Gut*, 67: 736-45.
- Cooper, S., A. L. Erickson, E. J. Adams, J. Kansopon, A. J. Weiner, D. Y. Chien, M. Houghton, P. Parham, and C. M. Walker. 1999. 'Analysis of a successful immune response against hepatitis C virus', *Immunity*, 10: 439-49.
- Crouchet, E., F. Wensch, C. Schuster, M. B. Zeisel, and T. F. Baumert. 2018. 'Host-targeting therapies for hepatitis C virus infection: current developments and future applications', *Therap Adv Gastroenterol*, 11: 1756284818759483.
- Daito, T., K. Watashi, A. Sluder, H. Ohashi, S. Nakajima, K. Borroto-Esoda, T. Fujita, and T. Wakita. 2014. 'Cyclophilin inhibitors reduce phosphorylation of RNA-dependent protein

- kinase to restore expression of IFN-stimulated genes in HCV-infected cells', *Gastroenterology*, 147: 463-72.
- Dao Thi, V. L., C. Granier, M. B. Zeisel, M. Guerin, J. Mancip, O. Granio, F. Penin, D. Lavillette, R. Bartenschlager, T. F. Baumert, F. L. Cosset, and M. Dreux. 2012. 'Characterization of hepatitis C virus particle subpopulations reveals multiple usage of the scavenger receptor BI for entry steps', *J Biol Chem*, 287: 31242-57.
- de Jong, Y. P., M. Dorner, M. C. Mommersteeg, J. W. Xiao, A. B. Balazs, J. B. Robbins, B. Y. Winer, S. Gerges, K. Vega, R. N. Labitt, B. M. Donovan, E. Giang, A. Krishnan, L. Chiriboga, M. R. Charlton, D. R. Burton, D. Baltimore, M. Law, C. M. Rice, and A. Ploss. 2014. 'Broadly neutralizing antibodies abrogate established hepatitis C virus infection', *Sci Transl Med*, 6: 254ra129.
- de Queiroz, Rafaela Muniz, Á%orika Carvalho, and Wagner Barbosa Dias. 2014. 'O-GlcNAcylation: The Sweet Side of the Cancer', *Frontiers in Oncology*, 4.
- Dennis, J. W., K. S. Lau, M. Demetriou, and I. R. Nabi. 2009. 'Adaptive regulation at the cell surface by N-glycosylation', *Traffic*, 10: 1569-78.
- Denolly, S., C. Granier, N. Fontaine, B. Pozzetto, T. Bourlet, M. Guerin, and F. L. Cosset. 2019. 'A serum protein factor mediates maturation and apoB-association of HCV particles in the extracellular milieu', *J Hepatol*, 70: 626-38.
- Diao, J., H. Pantua, H. Ngu, L. Komuves, L. Diehl, G. Schaefer, and S. B. Kapadia. 2012. 'Hepatitis C virus induces epidermal growth factor receptor activation via CD81 binding for viral internalization and entry', *J Virol*, 86: 10935-49.
- Dorfmueller, H. C., V. S. Borodkin, M. Schimpl, S. M. Shepherd, N. A. Shpiro, and D. M. van Aalten. 2006. 'GlcNAcstatin: a picomolar, selective O-GlcNAcase inhibitor that modulates intracellular O-glcNAcylation levels', *J Am Chem Soc*, 128: 16484-5.
- Dorner, M., J. A. Horwitz, B. M. Donovan, R. N. Labitt, W. C. Budell, T. Friling, A. Vogt, M. T. Catanese, T. Satoh, T. Kawai, S. Akira, M. Law, C. M. Rice, and A. Ploss. 2013. 'Completion of the entire hepatitis C virus life cycle in genetically humanized mice', *Nature*, 501: 237-41.
- Dorner, M., J. A. Horwitz, J. B. Robbins, W. T. Barry, Q. Feng, K. Mu, C. T. Jones, J. W. Schoggins, M. T. Catanese, D. R. Burton, M. Law, C. M. Rice, and A. Ploss. 2011. 'A genetically humanized mouse model for hepatitis C virus infection', *Nature*, 474: 208-11.
- Douam, F., V. L. Dao Thi, G. Maurin, J. Fresquet, D. Mompelat, M. B. Zeisel, T. F. Baumert, F. L. Cosset, and D. Lavillette. 2014. 'Critical interaction between E1 and E2 glycoproteins determines binding and fusion properties of hepatitis C virus during cell entry', *Hepatology*, 59: 776-88.
- Dreux, M., V. L. Dao Thi, J. Fresquet, M. Guerin, Z. Julia, G. Verney, D. Durantel, F. Zoulim, D. Lavillette, F. L. Cosset, and B. Bartosch. 2009. 'Receptor complementation and mutagenesis reveal SR-BI as an essential HCV entry factor and functionally imply its intra- and extra-cellular domains', *PLoS Pathog*, 5: e1000310.
- Duan, F., H. Wu, D. Jia, W. Wu, S. Ren, L. Wang, S. Song, X. Guo, F. Liu, Y. Ruan, and J. Gu. 2018. 'O-GlcNAcylation of RACK1 promotes hepatocellular carcinogenesis', *J Hepatol*, 68: 1191-202.
- Dubuisson, J. 2000. 'Folding, assembly and subcellular localization of hepatitis C virus glycoproteins', *Curr Top Microbiol Immunol*, 242: 135-48.
- Dubuisson, J., and F. L. Cosset. 2014. 'Virology and cell biology of the hepatitis C virus life cycle: an update', *J Hepatol*, 61: S3-S13.
- EASL. 2018. 'EASL Recommendations on Treatment of Hepatitis C 2018', *J Hepatol*, 69: 461-511.
- Edlin, B. R. 2016. 'Access to treatment for hepatitis C virus infection: time to put patients first', *Lancet Infect Dis*, 16: e196-e201.
- El-Serag, H. B., and J. A. Davila. 2011. 'Surveillance for hepatocellular carcinoma: in whom and how?', *Therap Adv Gastroenterol*, 4: 5-10.

- El-Serag, H. B., and K. L. Rudolph. 2007. 'Hepatocellular carcinoma: epidemiology and molecular carcinogenesis', *Gastroenterology*, 132: 2557-76.
- Esposito, I., J. Trinks, and V. Soriano. 2016. 'Hepatitis C virus resistance to the new direct-acting antivirals', *Expert Opin Drug Metab Toxicol*, 12: 1197-209.
- Evans, M. J., C. M. Rice, and S. P. Goff. 2004. 'Phosphorylation of hepatitis C virus nonstructural protein 5A modulates its protein interactions and viral RNA replication', *Proc Natl Acad Sci U S A*, 101: 13038-43.
- Evans, M. J., T. von Hahn, D. M. Tscherne, A. J. Syder, M. Panis, B. Wolk, T. Hatzioannou, J. A. McKeating, P. D. Bieniasz, and C. M. Rice. 2007. 'Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry', *Nature*, 446: 801-5.
- Fardini, Y., V. Dehennaut, T. Lefebvre, and T. Issad. 2013. 'O-GlcNAcylation: A New Cancer Hallmark?', *Front Endocrinol (Lausanne)*, 4: 99.
- Farquhar, M. J., H. J. Harris, M. Diskar, S. Jones, C. J. Mee, S. U. Nielsen, C. L. Brimacombe, S. Molina, G. L. Toms, P. Maurel, J. Howl, F. W. Herberg, S. C. van Ijzendoorn, P. Balfe, and J. A. McKeating. 2008. 'Protein kinase A-dependent step(s) in hepatitis C virus entry and infectivity', *J Virol*, 82: 8797-811.
- Farquhar, M. J., K. Hu, H. J. Harris, C. Davis, C. L. Brimacombe, S. J. Fletcher, T. F. Baumert, J. Z. Rappoport, P. Balfe, and J. A. McKeating. 2012. 'Hepatitis C virus induces CD81 and claudin-1 endocytosis', *J Virol*, 86: 4305-16.
- Fauvelle, C., D. J. Felmlee, E. Crouch, J. Lee, L. Heydmann, M. Lefevre, A. Magri, M. S. Hiet, I. Fofana, F. Habersetzer, S. K. Fong, R. Milne, A. H. Patel, K. Vercauteren, P. Meuleman, M. B. Zeisel, R. Bartenschlager, C. Schuster, and T. F. Baumert. 2016. 'Apolipoprotein E Mediates Evasion From Hepatitis C Virus Neutralizing Antibodies', *Gastroenterology*, 150: 206-17 e4.
- Felmlee, D. J., M. L. Hafirassou, M. Lefevre, T. F. Baumert, and C. Schuster. 2013. 'Hepatitis C virus, cholesterol and lipoproteins--impact for the viral life cycle and pathogenesis of liver disease', *Viruses*, 5: 1292-324.
- Felmlee, D. J., D. A. Sheridan, S. H. Bridge, S. U. Nielsen, R. W. Milne, C. J. Packard, M. J. Caslake, J. McLauchlan, G. L. Toms, R. D. Neely, and M. F. Bassendine. 2010. 'Intravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles', *Gastroenterology*, 139: 1774-83, 83.e1-6.
- Ferenci, P. 2015. 'Treatment of hepatitis C in difficult-to-treat patients', *Nat Rev Gastroenterol Hepatol*, 12: 284-92.
- Ferraris, P., E. Beaumont, R. Uzbekov, D. Brand, J. Gaillard, E. Blanchard, and P. Roingeard. 2013. 'Sequential biogenesis of host cell membrane rearrangements induced by hepatitis C virus infection', *Cell Mol Life Sci*, 70: 1297-306.
- Ferrer, C. M., T. Y. Lu, Z. A. Bacigalupa, C. D. Katsetos, D. A. Sinclair, and M. J. Reginato. 2017. 'O-GlcNAcylation regulates breast cancer metastasis via SIRT1 modulation of FOXM1 pathway', *Oncogene*, 36: 559-69.
- Ferrer, C. M., V. L. Sodi, and M. J. Reginato. 2016. 'O-GlcNAcylation in Cancer Biology: Linking Metabolism and Signaling', *J Mol Biol*, 428: 3282-94.
- Ferron, M., M. Denis, A. Persello, R. Rathagirishnan, and B. Lauzier. 2018. 'Protein O-GlcNAcylation in Cardiac Pathologies: Past, Present, Future', *Front Endocrinol (Lausanne)*, 9: 819.
- Fofana, I., S. E. Krieger, F. Grunert, S. Glauben, F. Xiao, S. Fafi-Kremer, E. Soulier, C. Royer, C. Thumann, C. J. Mee, J. A. McKeating, T. Dragic, P. Pessaux, F. Stoll-Keller, C. Schuster, J. Thompson, and T. F. Baumert. 2010. 'Monoclonal anti-claudin 1 antibodies prevent hepatitis C virus infection of primary human hepatocytes', *Gastroenterology*, 139: 953-64, 64 e1-4.
- Fofana, I., F. Xiao, C. Thumann, M. Turek, L. Zona, R. G. Tawar, F. Grunert, J. Thompson, M. B. Zeisel, and T. F. Baumert. 2013. 'A novel monoclonal anti-CD81 antibody produced by genetic immunization efficiently inhibits Hepatitis C virus cell-cell transmission', *PLoS One*, 8: e64221.

- Fournier, C., C. Sureau, J. Coste, J. Ducos, G. Pageaux, D. Larrey, J. Domergue, and P. Maurel. 1998. 'In vitro infection of adult normal human hepatocytes in primary culture by hepatitis C virus', *J Gen Virol*, 79 (Pt 10): 2367-74.
- Friebe, P., J. Boudet, J. P. Simorre, and R. Bartenschlager. 2005. 'Kissing-loop interaction in the 3' end of the hepatitis C virus genome essential for RNA replication', *J Virol*, 79: 380-92.
- Fuentes-Garcia, G., M. C. Castaneda-Patlan, A. S. Vercoutter-Edouart, T. Lefebvre, and M. Robles-Flores. 2019. 'O-GlcNAcylation Is Involved in the Regulation of Stem Cell Markers Expression in Colon Cancer Cells', *Front Endocrinol (Lausanne)*, 10: 289.
- Fujioka, Y., T. Taniguchi, Y. Ishikawa, M. Shiomi, and M. Yokoyama. 1994. 'Relation of N-glycosylation of apolipoprotein B-100 to cellular metabolism of low density lipoprotein', *Atherosclerosis*, 108: 91-102.
- Gastaminza, P., G. Cheng, S. Wieland, J. Zhong, W. Liao, and F. V. Chisari. 2008. 'Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion', *J Virol*, 82: 2120-9.
- Gastaminza, P., S. B. Kapadia, and F. V. Chisari. 2006. 'Differential biophysical properties of infectious intracellular and secreted hepatitis C virus particles', *J Virol*, 80: 11074-81.
- Ghosh, P., Q. H. Liu, and M. R. Lakshman. 1995. 'Long-term ethanol exposure impairs glycosylation of both N- and O-glycosylated proteins in rat liver', *Metabolism*, 44: 890-8.
- Gloster, Tracey M., Wesley F. Zandberg, Julia E. Heinonen, David L. Shen, Lehua Deng, and David J. Vocadlo. 2011. 'Hijacking a biosynthetic pathway yields a glycosyltransferase inhibitor within cells', *Nature Chemical Biology*, 7: 174-81.
- Goffard, A., N. Callens, B. Bartosch, C. Wychowski, F. L. Cosset, C. Montpellier, and J. Dubuisson. 2005. 'Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins', *J Virol*, 79: 8400-9.
- Gottwein, J. M., T. B. Jensen, C. K. Mathiesen, P. Meuleman, S. B. Serre, J. B. Lademann, L. Ghanem, T. K. Scheel, G. Leroux-Roels, and J. Bukh. 2011. 'Development and application of hepatitis C reporter viruses with genotype 1 to 7 core-nonstructural protein 2 (NS2) expressing fluorescent proteins or luciferase in modified JFH1 NS5A', *J Virol*, 85: 8913-28.
- Gottwein, J. M., T. K. Scheel, A. M. Hoegh, J. B. Lademann, J. Eugen-Olsen, G. Lisby, and J. Bukh. 2007. 'Robust hepatitis C genotype 3a cell culture releasing adapted intergenotypic 3a/2a (S52/JFH1) viruses', *Gastroenterology*, 133: 1614-26.
- Gottwein, J. M., T. K. Scheel, T. B. Jensen, J. B. Lademann, J. C. Prentoe, M. L. Knudsen, A. M. Hoegh, and J. Bukh. 2009. 'Development and characterization of hepatitis C virus genotype 1-7 cell culture systems: role of CD81 and scavenger receptor class B type I and effect of antiviral drugs', *Hepatology*, 49: 364-77.
- Gouttenoire, J., F. Penin, and D. Moradpour. 2010. 'Hepatitis C virus nonstructural protein 4B: a journey into unexplored territory', *Rev Med Virol*, 20: 117-29.
- Gower, E., C. Estes, S. Blach, K. Razavi-Shearer, and H. Razavi. 2014. 'Global epidemiology and genotype distribution of the hepatitis C virus infection', *J Hepatol*, 61: S45-57.
- Grakoui, A., D. W. McCourt, C. Wychowski, S. M. Feinstone, and C. M. Rice. 1993. 'A second hepatitis C virus-encoded proteinase', *Proc Natl Acad Sci U S A*, 90: 10583-7.
- Grigorov, B., E. Reungoat, A. Gentil Dit Maurin, M. Varbanov, J. Blaising, M. Michelet, R. Manuel, R. Parent, B. Bartosch, F. Zoulim, F. Ruggiero, and E. I. Pecheur. 2017. 'Hepatitis C virus infection propagates through interactions between Syndecan-1 and CD81 and impacts the hepatocyte glycocalyx', *Cell Microbiol*, 19.
- Grompe, M., M. al-Dhalimy, M. Finegold, C. N. Ou, T. Burlingame, N. G. Kennaway, and P. Soriano. 1993. 'Loss of fumarylacetoacetate hydrolase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice', *Genes Dev*, 7: 2298-307.
- Groussaud, D., M. Khair, A. I. Tollenaere, L. Waast, M. S. Kuo, M. Mangeney, C. Martella, Y. Fardini, S. Coste, M. Souidi, L. Benit, C. Pique, and T. Issad. 2017. 'Hijacking of the O-GlcNAcZYME complex by the HTLV-1 Tax oncoprotein facilitates viral transcription', *PLoS Pathog*, 13: e1006518.

- Gu, Y., W. Mi, Y. Ge, H. Liu, Q. Fan, C. Han, J. Yang, F. Han, X. Lu, and W. Yu. 2010. 'GlcNAcylation plays an essential role in breast cancer metastasis', *Cancer Res*, 70: 6344-51.
- Haddad, J. G., Y. Rouille, X. Hanouille, V. Descamps, M. Hamze, F. Dabboussi, T. F. Baumert, G. Duverlie, M. Lavie, and J. Dubuisson. 2017. 'Identification of Novel Functions for Hepatitis C Virus Envelope Glycoprotein E1 in Virus Entry and Assembly', *J Virol*, 91.
- Haid, S., T. Pietschmann, and E. I. Pecheur. 2009. 'Low pH-dependent hepatitis C virus membrane fusion depends on E2 integrity, target lipid composition, and density of virus particles', *J Biol Chem*, 284: 17657-67.
- Hamdane, N., F. Juhling, E. Crouchet, H. El Saghire, C. Thumann, M. A. Oudot, S. Bandiera, A. Saviano, C. Ponsolles, A. A. Roca Suarez, S. Li, N. Fujiwara, A. Ono, I. Davidson, N. Bardeesy, C. Schmidl, C. Bock, C. Schuster, J. Lupberger, F. Habersetzer, M. Doffoel, T. Piardi, D. Sommacale, M. Imamura, T. Uchida, H. Ohdan, H. Aikata, K. Chayama, T. Boldanova, P. Pessaux, B. C. Fuchs, Y. Hoshida, M. B. Zeisel, F. H. T. Duong, and T. F. Baumert. 2019. 'HCV-Induced Epigenetic Changes Associated With Liver Cancer Risk Persist After Sustained Virologic Response', *Gastroenterology*, 156: 2313-29 e7.
- Harris, H. J., C. Davis, J. G. Mullins, K. Hu, M. Goodall, M. J. Farquhar, C. J. Mee, K. McCaffrey, S. Young, H. Drummer, P. Balfe, and J. A. McKeating. 2010. 'Claudin association with CD81 defines hepatitis C virus entry', *J Biol Chem*, 285: 21092-102.
- Harris, H. J., M. J. Farquhar, C. J. Mee, C. Davis, G. M. Reynolds, A. Jennings, K. Hu, F. Yuan, H. Deng, S. G. Hubscher, J. H. Han, P. Balfe, and J. A. McKeating. 2008. 'CD81 and claudin 1 coreceptor association: role in hepatitis C virus entry', *J Virol*, 82: 5007-20.
- Hart, G. W., K. D. Greis, L. Y. Dong, M. A. Blomberg, T. Y. Chou, M. S. Jiang, E. P. Roquemore, D. M. Snow, L. K. Kreppel, R. N. Cole, and et al. 1995. 'O-linked N-acetylglucosamine: the "yin-yang" of Ser/Thr phosphorylation? Nuclear and cytoplasmic glycosylation', *Adv Exp Med Biol*, 376: 115-23.
- Hart, G. W., M. P. Housley, and C. Slawson. 2007. 'Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins', *Nature*, 446: 1017-22.
- Hart, G. W., C. Slawson, G. Ramirez-Correa, and O. Lagerlof. 2011. 'Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease', *Annu Rev Biochem*, 80: 825-58.
- Helenius, A., and M. Aebi. 2001. 'Intracellular functions of N-linked glycans', *Science*, 291: 2364-9.
- Helle, F., G. Duverlie, and J. Dubuisson. 2011. 'The hepatitis C virus glycan shield and evasion of the humoral immune response', *Viruses*, 3: 1909-32.
- Helle, F., A. Goffard, V. Morel, G. Duverlie, J. McKeating, Z. Y. Keck, S. Fong, F. Penin, J. Dubuisson, and C. Voisset. 2007. 'The neutralizing activity of anti-hepatitis C virus antibodies is modulated by specific glycans on the E2 envelope protein', *J Virol*, 81: 8101-11.
- Henke, J. I., D. Goergen, J. Zheng, Y. Song, C. G. Schuttler, C. Fehr, C. Junemann, and M. Niepmann. 2008. 'microRNA-122 stimulates translation of hepatitis C virus RNA', *EMBO J*, 27: 3300-10.
- Herker, E., C. Harris, C. Hernandez, A. Carpentier, K. Kaehleke, A. R. Rosenberg, R. V. Farese, Jr., and M. Ott. 2010. 'Efficient hepatitis C virus particle formation requires diacylglycerol acyltransferase-1', *Nat Med*, 16: 1295-8.
- Herzog, K., S. Bandiera, S. Pernot, C. Fauvelle, F. Juhling, A. Weiss, A. Bull, S. C. Durand, B. Chane-Woon-Ming, S. Pfeffer, M. Mercey, H. Lerat, J. C. Meunier, W. Raffelsberger, L. Brino, T. F. Baumert, and M. B. Zeisel. 2019. 'Functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as a host factor modulating hepatitis C virus morphogenesis and infectivity', *Gut*.
- Hezode, C., H. Fontaine, C. Dorival, D. Larrey, F. Zoulim, V. Canva, V. de Ledinghen, T. Poynard, D. Samuel, M. Bourliere, J. P. Zarski, J. J. Raabe, L. Alric, P. Marcellin, G. Riachi, P. H. Bernard, V. Loustaud-Ratti, S. Metivier, A. Tran, L. Serfaty, A. Abergel, X. Causse, V. Di Martino, D. Guyader, D. Lucidarme, V. Grando-Lemaire, P. Hillon, C. Feray, T. Dao, P.

- Cacoub, I. Rosa, P. Attali, V. Petrov-Sanchez, Y. Barthe, J. M. Pawlotsky, S. Pol, F. Carrat, and J. P. Bronowicki. 2013. 'Triple therapy in treatment-experienced patients with HCV-cirrhosis in a multicentre cohort of the French Early Access Programme (ANRS CO20-CUPIC) - NCT01514890', *J Hepatol*, 59: 434-41.
- Hijikata, M., Y. K. Shimizu, H. Kato, A. Iwamoto, J. W. Shih, H. J. Alter, R. H. Purcell, and H. Yoshikura. 1993. 'Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes', *J Virol*, 67: 1953-8.
- Hofmann, S., M. Krajewski, C. Scherer, V. Scholz, V. Mordhorst, P. Truschow, A. Schobel, R. Reimer, D. Schwudke, and E. Herker. 2018. 'Complex lipid metabolic remodeling is required for efficient hepatitis C virus replication', *Biochim Biophys Acta Mol Cell Biol Lipids*, 1863: 1041-56.
- Hoofnagle, J. H., and A. M. di Bisceglie. 1997. 'The treatment of chronic viral hepatitis', *N Engl J Med*, 336: 347-56.
- Hopkins, S., M. Bobardt, U. Chatterji, J. A. Garcia-Rivera, P. Lim, and P. A. Gallay. 2012. 'The cyclophilin inhibitor SCY-635 disrupts hepatitis C virus NS5A-cyclophilin A complexes', *Antimicrob Agents Chemother*, 56: 3888-97.
- Hsu, M., J. Zhang, M. Flint, C. Logvinoff, C. Cheng-Mayer, C. M. Rice, and J. A. McKeating. 2003. 'Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles', *Proc Natl Acad Sci U S A*, 100: 7271-6.
- Huang, H., F. Sun, D. M. Owen, W. Li, Y. Chen, M. Gale, Jr., and J. Ye. 2007. 'Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins', *Proc Natl Acad Sci U S A*, 104: 5848-53.
- Hundt, J., Z. Li, and Q. Liu. 2013. 'Post-translational modifications of hepatitis C viral proteins and their biological significance', *World J Gastroenterol*, 19: 8929-39.
- Issad, T., E. Masson, and P. Pagesy. 2010. 'O-GlcNAc modification, insulin signaling and diabetic complications', *Diabetes Metab*, 36: 423-35.
- Itkonen, H. M., S. S. Gorad, D. Y. Duveau, S. E. Martin, A. Barkovskaya, T. F. Batten, S. A. Moestue, and I. G. Mills. 2016. 'Inhibition of O-GlcNAc transferase activity reprograms prostate cancer cell metabolism', *Oncotarget*, 7: 12464-76.
- Itkonen, H. M., S. Minner, I. J. Guldvik, M. J. Sandmann, M. C. Tsourlakis, V. Berge, A. Svindland, T. Schlomm, and I. G. Mills. 2013. 'O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer cells', *Cancer Res*, 73: 5277-87.
- Itkonen, H. M., A. Urbanucci, S. E. Martin, A. Khan, A. Mathelier, B. Thiede, S. Walker, and I. G. Mills. 2019. 'High OGT activity is essential for MYC-driven proliferation of prostate cancer cells', *Theranostics*, 9: 2183-97.
- Iyengar, S., K. Tay-Teo, S. Vogler, P. Beyer, S. Wiktor, K. de Joncheere, and S. Hill. 2016. 'Prices, Costs, and Affordability of New Medicines for Hepatitis C in 30 Countries: An Economic Analysis', *PLoS Med*, 13: e1002032.
- Jacobson, I. M., J. G. McHutchison, G. Dusheiko, A. M. Di Bisceglie, K. R. Reddy, N. H. Bzowej, P. Marcellin, A. J. Muir, P. Ferenci, R. Flisiak, J. George, M. Rizzetto, D. Shouval, R. Sola, R. A. Terg, E. M. Yoshida, N. Adda, L. Bengtsson, A. J. Sankoh, T. L. Kieffer, S. George, R. S. Kauffman, and S. Zeuzem. 2011. 'Telaprevir for previously untreated chronic hepatitis C virus infection', *N Engl J Med*, 364: 2405-16.
- Jensen, T. B., J. M. Gottwein, T. K. Scheel, A. M. Hoegh, J. Eugen-Olsen, and J. Bukh. 2008. 'Highly efficient JFH1-based cell-culture system for hepatitis C virus genotype 5a: failure of homologous neutralizing-antibody treatment to control infection', *J Infect Dis*, 198: 1756-65.
- Jiang, J., W. Cun, X. Wu, Q. Shi, H. Tang, and G. Luo. 2012. 'Hepatitis C virus attachment mediated by apolipoprotein E binding to cell surface heparan sulfate', *J Virol*, 86: 7256-67.
- Jiang, J., M. B. Lazarus, L. Pasquina, P. Sliz, and S. Walker. 2011. 'A neutral diphosphate mimic crosslinks the active site of human O-GlcNAc transferase', *Nat Chem Biol*, 8: 72-7.

- Jiang, J., and G. Luo. 2009. 'Apolipoprotein E but not B is required for the formation of infectious hepatitis C virus particles', *J Virol*, 83: 12680-91.
- Jirasko, V., R. Montserret, J. Y. Lee, J. Gouttenoire, D. Moradpour, F. Penin, and R. Bartenschlager. 2010. 'Structural and functional studies of nonstructural protein 2 of the hepatitis C virus reveal its key role as organizer of virion assembly', *PLoS Pathog*, 6: e1001233.
- Jochmann, R., J. Pfannstiel, P. Chudasama, E. Kuhn, A. Konrad, and M. Sturzl. 2013. 'O-GlcNAc transferase inhibits KSHV propagation and modifies replication relevant viral proteins as detected by systematic O-GlcNAcylation analysis', *Glycobiology*, 23: 1114-30.
- Jochmann, R., M. Thureau, S. Jung, C. Hofmann, E. Naschberger, E. Kremmer, T. Harrer, M. Miller, N. Schaft, and M. Sturzl. 2009. 'O-linked N-acetylglucosamylation of Sp1 inhibits the human immunodeficiency virus type 1 promoter', *J Virol*, 83: 3704-18.
- Johansson, D. X., C. Voisset, A. W. Tarr, M. Aung, J. K. Ball, J. Dubuisson, and M. A. Persson. 2007. 'Human combinatorial libraries yield rare antibodies that broadly neutralize hepatitis C virus', *Proc Natl Acad Sci U S A*, 104: 16269-74.
- Joiner, C. M., H. Li, J. Jiang, and S. Walker. 2019. 'Structural characterization of the O-GlcNAc cycling enzymes: insights into substrate recognition and catalytic mechanisms', *Curr Opin Struct Biol*, 56: 97-106.
- Jones, C. T., M. T. Catanese, L. M. Law, S. R. Khetani, A. J. Syder, A. Ploss, T. S. Oh, J. W. Schoggins, M. R. MacDonald, S. N. Bhatia, and C. M. Rice. 2010. 'Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system', *Nat Biotechnol*, 28: 167-71.
- Jopling, C. L., S. Schutz, and P. Sarnow. 2008. 'Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome', *Cell Host Microbe*, 4: 77-85.
- Jopling, C. L., M. Yi, A. M. Lancaster, S. M. Lemon, and P. Sarnow. 2005. 'Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA', *Science*, 309: 1577-81.
- Kamigaito, T., T. Okaneya, M. Kawakubo, H. Shimojo, O. Nishizawa, and J. Nakayama. 2014. 'Overexpression of O-GlcNAc by prostate cancer cells is significantly associated with poor prognosis of patients', *Prostate Cancer Prostatic Dis*, 17: 18-22.
- Kanwal, F., J. R. Kramer, S. M. Asch, Y. Cao, L. Li, and H. B. El-Serag. 2019. 'Long-Term Risk of Hepatocellular Carcinoma in HCV Patients Treated With Direct Acting Antiviral Agents', *Hepatology*.
- Kapadia, S. B., H. Barth, T. Baumert, J. A. McKeating, and F. V. Chisari. 2007. 'Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I', *J Virol*, 81: 374-83.
- Kato, T., A. Furusaka, M. Miyamoto, T. Date, K. Yasui, J. Hiramoto, K. Nagayama, T. Tanaka, and T. Wakita. 2001. 'Sequence analysis of hepatitis C virus isolated from a fulminant hepatitis patient', *J Med Virol*, 64: 334-9.
- Kaul, A., S. Stauffer, C. Berger, T. Pertel, J. Schmitt, S. Kallis, M. Zayas, V. Lohmann, J. Luban, and R. Bartenschlager. 2009. 'Essential role of cyclophilin A for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics', *PLoS Pathog*, 5: e1000546.
- Kim, M., Y. S. Kim, H. Kim, M. Y. Kang, J. Park, D. H. Lee, G. S. Roh, H. J. Kim, S. S. Kang, G. J. Cho, J. K. Park, J. W. Cho, J. K. Shin, and W. S. Choi. 2016. 'O-linked N-acetylglucosamine transferase promotes cervical cancer tumorigenesis through human papillomaviruses E6 and E7 oncogenes', *Oncotarget*, 7: 44596-607.
- Krzyslak, A., P. Jozwiak, and A. Lipinska. 2011. 'Down-regulation of beta-N-acetyl-D-glucosaminidase increases Akt1 activity in thyroid anaplastic cancer cells', *Oncol Rep*, 26: 743-9.

- Krzeslak, A., K. Wojcik-Krowiranda, E. Forma, A. Bienkiewicz, and M. Brys. 2012. 'Expression of genes encoding for enzymes associated with O-GlcNAcylation in endometrial carcinomas: clinicopathologic correlations', *Ginekol Pol*, 83: 22-6.
- Lacek, K., K. Vercauteren, K. Grzyb, M. Naddeo, L. Verhoye, M. P. Slowikowski, S. Fafi-Kremer, A. H. Patel, T. F. Baumert, A. Folgori, G. Leroux-Roels, R. Cortese, P. Meuleman, and A. Nicosia. 2012. 'Novel human SR-BI antibodies prevent infection and dissemination of HCV in vitro and in humanized mice', *J Hepatol*, 57: 17-23.
- Lavie, M., and J. Dubuisson. 2017. 'Interplay between hepatitis C virus and lipid metabolism during virus entry and assembly', *Biochimie*, 141: 62-69.
- Lavie, M., X. Hanouille, and J. Dubuisson. 2018. 'Glycan Shielding and Modulation of Hepatitis C Virus Neutralizing Antibodies', *Front Immunol*, 9: 910.
- Lavillette, D., B. Bartosch, D. Nourrisson, G. Verney, F. L. Cosset, F. Penin, and E. I. Pecheur. 2006. 'Hepatitis C virus glycoproteins mediate low pH-dependent membrane fusion with liposomes', *J Biol Chem*, 281: 3909-17.
- Lazarus, B. D., D. C. Love, and J. A. Hanover. 2006. 'Recombinant O-GlcNAc transferase isoforms: identification of O-GlcNAcase, yes tyrosine kinase, and tau as isoform-specific substrates', *Glycobiology*, 16: 415-21.
- Lee, K. Y., Y. H. Chen, S. C. Hsu, and M. J. Yu. 2016. 'Phosphorylation of Serine 235 of the Hepatitis C Virus Non-Structural Protein NS5A by Multiple Kinases', *PLoS One*, 11: e0166763.
- Lee, T. N., W. E. Alborn, M. D. Knierman, and R. J. Konrad. 2006. 'Alloxan is an inhibitor of O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase', *Biochem Biophys Res Commun*, 350: 1038-43.
- Lee, Y., M. Kockx, M. J. Raftery, W. Jessup, R. Griffith, and L. Kritharides. 2010. 'Glycosylation and sialylation of macrophage-derived human apolipoprotein E analyzed by SDS-PAGE and mass spectrometry: evidence for a novel site of glycosylation on Ser290', *Mol Cell Proteomics*, 9: 1968-81.
- Lefevre, M., D. J. Felmlee, M. Parnot, T. F. Baumert, and C. Schuster. 2014. 'Syndecan 4 is involved in mediating HCV entry through interaction with lipoviral particle-associated apolipoprotein E', *PLoS One*, 9: e95550.
- Leney, A. C., D. El Atmioui, W. Wu, H. Ovaa, and A. J. R. Heck. 2017. 'Elucidating crosstalk mechanisms between phosphorylation and O-GlcNAcylation', *Proc Natl Acad Sci U S A*, 114: E7255-e61.
- Levin, A., C. J. Neufeldt, D. Pang, K. Wilson, D. Loewen-Dobler, M. A. Joyce, R. W. Wozniak, and D. L. Tyrrell. 2014. 'Functional characterization of nuclear localization and export signals in hepatitis C virus proteins and their role in the membranous web', *PLoS One*, 9: e114629.
- Levine, Z. G., and S. Walker. 2016. 'The Biochemistry of O-GlcNAc Transferase: Which Functions Make It Essential in Mammalian Cells?', *Annu Rev Biochem*, 85: 631-57.
- Li, G., and E. De Clercq. 2017. 'Current therapy for chronic hepatitis C: The role of direct-acting antivirals', *Antiviral Res*, 142: 83-122.
- Li, H., J. D. Jiang, and Z. G. Peng. 2016. 'MicroRNA-mediated interactions between host and hepatitis C virus', *World J Gastroenterol*, 22: 1487-96.
- Li, Q., B. Lowey, C. Sodroski, S. Krishnamurthy, H. Alao, H. Cha, S. Chiu, R. El-Diwany, M. G. Ghany, and T. J. Liang. 2017. 'Cellular microRNA networks regulate host dependency of hepatitis C virus infection', *Nat Commun*, 8: 1789.
- Li, Y. P., J. M. Gottwein, T. K. Scheel, T. B. Jensen, and J. Bukh. 2011. 'MicroRNA-122 antagonism against hepatitis C virus genotypes 1-6 and reduced efficacy by host RNA insertion or mutations in the HCV 5' UTR', *Proc Natl Acad Sci U S A*, 108: 4991-6.
- Li, Y., D. Yamane, T. Masaki, and S. M. Lemon. 2015. 'The yin and yang of hepatitis C: synthesis and decay of hepatitis C virus RNA', *Nat Rev Microbiol*, 13: 544-58.

- Liberti, M. V., and J. W. Locasale. 2016. 'The Warburg Effect: How Does it Benefit Cancer Cells?', *Trends Biochem Sci*, 41: 211-18.
- Lin, Y. C., C. H. Lin, Y. C. Yeh, H. L. Ho, Y. C. Wu, M. Y. Chen, and T. Y. Chou. 2018. 'High O-linked N-acetylglucosamine transferase expression predicts poor survival in patients with early stage lung adenocarcinoma', *Oncotarget*, 9: 31032-44.
- Lindenbach, B. D. 2013. 'Virion assembly and release', *Curr Top Microbiol Immunol*, 369: 199-218.
- Lindenbach, B. D., M. J. Evans, A. J. Syder, B. Wolk, T. L. Tellinghuisen, C. C. Liu, T. Maruyama, R. O. Hynes, D. R. Burton, J. A. McKeating, and C. M. Rice. 2005. 'Complete replication of hepatitis C virus in cell culture', *Science*, 309: 623-6.
- Lindenbach, B. D., P. Meuleman, A. Ploss, T. Vanwolleghem, A. J. Syder, J. A. McKeating, R. E. Lanford, S. M. Feinstone, M. E. Major, G. Leroux-Roels, and C. M. Rice. 2006. 'Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro', *Proc Natl Acad Sci U S A*, 103: 3805-9.
- Lindenbach, B. D., and C. M. Rice. 2013. 'The ins and outs of hepatitis C virus entry and assembly', *Nat Rev Microbiol*, 11: 688-700.
- Liu, S., W. Yang, L. Shen, J. R. Turner, C. B. Coyne, and T. Wang. 2009. 'Tight junction proteins claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection', *J Virol*, 83: 2011-4.
- Liu, T. W., W. F. Zandberg, T. M. Gloster, L. Deng, K. D. Murray, X. Shan, and D. J. Vocadlo. 2018. 'Metabolic Inhibitors of O-GlcNAc Transferase That Act In Vivo Implicate Decreased O-GlcNAc Levels in Leptin-Mediated Nutrient Sensing', *Angew Chem Int Ed Engl*, 57: 7644-48.
- Liu, Y., H. Huang, Y. Cao, Q. Wu, W. Li, and J. Zhang. 2017. 'Suppression of OGT by microRNA24 reduces FOXA1 stability and prevents breast cancer cells invasion', *Biochem Biophys Res Commun*, 487: 755-62.
- Liu, Yan, Shaojun Dai, Lijing Xing, Yunyuan Xu, and Kang Chong. 2015. 'O-linked β -N-acetylglucosamine modification and its biological functions', *Science Bulletin*, 60: 1055-61.
- Liu, Z., F. Yang, J. M. Robotham, and H. Tang. 2009. 'Critical role of cyclophilin A and its prolyl-peptidyl isomerase activity in the structure and function of the hepatitis C virus replication complex', *J Virol*, 83: 6554-65.
- Lohmann, V. 2013. 'Hepatitis C virus RNA replication', *Curr Top Microbiol Immunol*, 369: 167-98.
- Lohmann, V., F. Korner, U. Herian, and R. Bartenschlager. 1997. 'Biochemical properties of hepatitis C virus NS5B RNA-dependent RNA polymerase and identification of amino acid sequence motifs essential for enzymatic activity', *J Virol*, 71: 8416-28.
- Lohmann, V., F. Korner, J. Koch, U. Herian, L. Theilmann, and R. Bartenschlager. 1999. 'Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line', *Science*, 285: 110-3.
- Lukavsky, P. J. 2009. 'Structure and function of HCV IRES domains', *Virus Res*, 139: 166-71.
- Luo, C., D. Yin, H. Zhan, U. Borjigin, C. Li, Z. Zhou, Z. Hu, P. Wang, Q. Sun, J. Fan, J. Zhou, X. Wang, S. Zhou, and X. Huang. 2018. 'microRNA-501-3p suppresses metastasis and progression of hepatocellular carcinoma through targeting LIN7A', *Cell Death Dis*, 9: 535.
- Lupberger, J., M. B. Zeisel, F. Xiao, C. Thumann, I. Fofana, L. Zona, C. Davis, C. J. Mee, M. Turek, S. Gorke, C. Royer, B. Fischer, M. N. Zahid, D. Lavillette, J. Fresquet, F. L. Cosset, S. M. Rothenberg, T. Pietschmann, A. H. Patel, P. Pessaux, M. Doffoel, W. Raffelsberger, O. Poch, J. A. McKeating, L. Brino, and T. F. Baumert. 2011. 'EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy', *Nat Med*, 17: 589-95.
- Lynch, T. P., C. M. Ferrer, S. R. Jackson, K. S. Shahriari, K. Vosseller, and M. J. Reginato. 2012. 'Critical role of O-Linked beta-N-acetylglucosamine transferase in prostate cancer invasion, angiogenesis, and metastasis', *J Biol Chem*, 287: 11070-81.

- Ma, Y., M. Anantpadma, J. M. Timpe, S. Shanmugam, S. M. Singh, S. M. Lemon, and M. Yi. 2011. 'Hepatitis C virus NS2 protein serves as a scaffold for virus assembly by interacting with both structural and nonstructural proteins', *J Virol*, 85: 86-97.
- Ma, Z., D. J. Vocadlo, and K. Vosseller. 2013. 'Hyper-O-GlcNAcylation is anti-apoptotic and maintains constitutive NF-kappaB activity in pancreatic cancer cells', *J Biol Chem*, 288: 15121-30.
- Maillard, P., T. Huby, U. Andreo, M. Moreau, J. Chapman, and A. Budkowska. 2006. 'The interaction of natural hepatitis C virus with human scavenger receptor SR-BI/Cla1 is mediated by ApoB-containing lipoproteins', *Faseb j*, 20: 735-7.
- Maily, L., F. Xiao, J. Lupberger, G. K. Wilson, P. Aubert, F. H. T. Duong, D. Calabrese, C. Leboeuf, I. Fofana, C. Thumann, S. Bandiera, M. Lutgehetmann, T. Volz, C. Davis, H. J. Harris, C. J. Mee, E. Girardi, B. Chane-Woon-Ming, M. Ericsson, N. Fletcher, R. Bartenschlager, P. Pessaux, K. Vercauteren, P. Meuleman, P. Villa, L. Kaderali, S. Pfeffer, M. H. Heim, M. Neunlist, M. B. Zeisel, M. Dandri, J. A. McKeating, E. Robinet, and T. F. Baumert. 2015. 'Clearance of persistent hepatitis C virus infection in humanized mice using a claudin-1-targeting monoclonal antibody', *Nat Biotechnol*, 33: 549-54.
- Major, M. E., H. Dahari, K. Mihalik, M. Puig, C. M. Rice, A. U. Neumann, and S. M. Feinstone. 2004. 'Hepatitis C virus kinetics and host responses associated with disease and outcome of infection in chimpanzees', *Hepatology*, 39: 1709-20.
- Majzoub, K., M. L. Hafirassou, C. Meignin, A. Goto, S. Marzi, A. Fedorova, Y. Verdier, J. Vinh, J. A. Hoffmann, F. Martin, T. F. Baumert, C. Schuster, and J. L. Imler. 2014. 'RACK1 controls IRES-mediated translation of viruses', *Cell*, 159: 1086-95.
- Manns, M. P., J. G. McHutchison, S. C. Gordon, V. K. Rustgi, M. Shiffman, R. Reindollar, Z. D. Goodman, K. Koury, M. Ling, and J. K. Albrecht. 2001. 'Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial', *Lancet*, 358: 958-65.
- Manns, M. P., H. Wedemeyer, and M. Cornberg. 2006. 'Treating viral hepatitis C: efficacy, side effects, and complications', *Gut*, 55: 1350-9.
- Mariappa, D., K. Sauert, K. Marino, D. Turnock, R. Webster, D. M. van Aalten, M. A. Ferguson, and H. A. Muller. 2011. 'Protein O-GlcNAcylation is required for fibroblast growth factor signaling in Drosophila', *Sci Signal*, 4: ra89.
- Masaki, T., S. Matsunaga, H. Takahashi, K. Nakashima, Y. Kimura, M. Ito, M. Matsuda, A. Murayama, T. Kato, H. Hirano, Y. Endo, S. M. Lemon, T. Wakita, T. Sawasaki, and T. Suzuki. 2014. 'Involvement of hepatitis C virus NS5A hyperphosphorylation mediated by casein kinase I-alpha in infectious virus production', *J Virol*, 88: 7541-55.
- Matto, M., C. M. Rice, B. Aroeti, and J. S. Glenn. 2004. 'Hepatitis C virus core protein associates with detergent-resistant membranes distinct from classical plasma membrane rafts', *J Virol*, 78: 12047-53.
- McHutchison, J. G., S. C. Gordon, E. R. Schiff, M. L. Shiffman, W. M. Lee, V. K. Rustgi, Z. D. Goodman, M. H. Ling, S. Cort, and J. K. Albrecht. 1998. 'Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group', *N Engl J Med*, 339: 1485-92.
- McLauchlan, J., M. K. Lemberg, G. Hope, and B. Martoglio. 2002. 'Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets', *EMBO J*, 21: 3980-8.
- Mercer, D. F., D. E. Schiller, J. F. Elliott, D. N. Douglas, C. Hao, A. Rinfret, W. R. Addison, K. P. Fischer, T. A. Churchill, J. R. Lakey, D. L. Tyrrell, and N. M. Kneteman. 2001. 'Hepatitis C virus replication in mice with chimeric human livers', *Nat Med*, 7: 927-33.
- Merz, A., G. Long, M. S. Hiet, B. Brugger, P. Chlanda, P. Andre, F. Wieland, J. Krijnse-Locker, and R. Bartenschlager. 2011. 'Biochemical and morphological properties of hepatitis C virus particles and determination of their lipidome', *J Biol Chem*, 286: 3018-32.
- Meuleman, P., M. T. Catanese, L. Verhoye, I. Desombere, A. Farhoudi, C. T. Jones, T. Sheahan, K. Grzyb, R. Cortese, C. M. Rice, G. Leroux-Roels, and A. Nicosia. 2012. 'A human

- monoclonal antibody targeting scavenger receptor class B type I precludes hepatitis C virus infection and viral spread in vitro and in vivo', *Hepatology*, 55: 364-72.
- Meunier, J. C., R. S. Russell, R. E. Engle, K. N. Faulk, R. H. Purcell, and S. U. Emerson. 2008. 'Apolipoprotein c1 association with hepatitis C virus', *J Virol*, 82: 9647-56.
- Mi, W., Y. Gu, C. Han, H. Liu, Q. Fan, X. Zhang, Q. Cong, and W. Yu. 2011. 'O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy', *Biochim Biophys Acta*, 1812: 514-9.
- Michalopoulos, G. K., and M. C. DeFrances. 1997. 'Liver regeneration', *Science*, 276: 60-6.
- Miller, S., and J. Krijnse-Locker. 2008. 'Modification of intracellular membrane structures for virus replication', *Nat Rev Microbiol*, 6: 363-74.
- Miyanari, Y., K. Atsuzawa, N. Usuda, K. Watashi, T. Hishiki, M. Zayas, R. Bartenschlager, T. Wakita, M. Hijikata, and K. Shimotohno. 2007. 'The lipid droplet is an important organelle for hepatitis C virus production', *Nat Cell Biol*, 9: 1089-97.
- Molina, S., V. Castet, L. Pichard-Garcia, C. Wychowski, E. Meurs, J. M. Pascussi, C. Sureau, J. M. Fabre, A. Sacunha, D. Larrey, J. Dubuisson, J. Coste, J. McKeating, P. Maurel, and C. Fournier-Wirth. 2008. 'Serum-derived hepatitis C virus infection of primary human hepatocytes is tetraspanin CD81 dependent', *J Virol*, 82: 569-74.
- Morgan, R. L., B. Baack, B. D. Smith, A. Yartel, M. Pitasi, and Y. Falck-Ytter. 2013. 'Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies', *Ann Intern Med*, 158: 329-37.
- Nakabayashi, H., K. Taketa, K. Miyano, T. Yamane, and J. Sato. 1982. 'Growth of human hepatoma cells lines with differentiated functions in chemically defined medium', *Cancer Res*, 42: 3858-63.
- Naoumov, N. V. 2014. 'Cyclophilin inhibition as potential therapy for liver diseases', *J Hepatol*, 61: 1166-74.
- National Research Council Committee on the Use of Chimpanzees in, Biomedical, and Research Behavioral. 2011. 'The National Academies Collection: Reports funded by National Institutes of Health.' in B. M. Altevogt, D. E. Pankevich, M. K. Shelton-Davenport and J. P. Kahn (eds.), *Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity* (National Academies Press (US))
- National Academy of Sciences.: Washington (DC)).
- Neufeldt, C. J., M. A. Joyce, A. Levin, R. H. Steenbergen, D. Pang, J. Shields, D. L. Tyrrell, and R. W. Wozniak. 2013. 'Hepatitis C virus-induced cytoplasmic organelles use the nuclear transport machinery to establish an environment conducive to virus replication', *PLoS Pathog*, 9: e1003744.
- Nie, H., and W. Yi. 2019. 'O-GlcNAcylation, a sweet link to the pathology of diseases', *J Zhejiang Univ Sci B*, 20: 437-48.
- Nielsen, S. U., M. F. Bassendine, A. D. Burt, D. J. Bevitt, and G. L. Toms. 2004. 'Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver', *J Gen Virol*, 85: 1497-507.
- Nielsen, S. U., M. F. Bassendine, A. D. Burt, C. Martin, W. Pumeechockchai, and G. L. Toms. 2006. 'Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients', *J Virol*, 80: 2418-28.
- Niepmann, M., L. A. Shalamova, G. K. Gerresheim, and O. Rossbach. 2018. 'Signals Involved in Regulation of Hepatitis C Virus RNA Genome Translation and Replication', *Front Microbiol*, 9: 395.
- NIH. 2002. 'Consensus Statement on Management of Hepatitis C', *NIH Consens State Sci Statements*, 19: 1-46.
- Olofsson, S. O., P. Bostrom, L. Andersson, M. Rutberg, J. Perman, and J. Boren. 2009. 'Lipid droplets as dynamic organelles connecting storage and efflux of lipids', *Biochim Biophys Acta*, 1791: 448-58.

- Ortiz-Meoz, R. F., J. Jiang, M. B. Lazarus, M. Orman, J. Janetzko, C. Fan, D. Y. Dubeau, Z. W. Tan, C. J. Thomas, and S. Walker. 2015. 'A small molecule that inhibits OGT activity in cells', *ACS Chem Biol*, 10: 1392-7.
- Paciello, R., R. A. Urbanowicz, G. Riccio, E. Sasso, C. P. McClure, N. Zambrano, J. K. Ball, R. Cortese, A. Nicosia, and C. De Lorenzo. 2016. 'Novel human anti-claudin 1 mAbs inhibit hepatitis C virus infection and may synergize with anti-SRB1 mAb', *J Gen Virol*, 97: 82-94.
- Pantua, H., J. Diao, M. Ultsch, M. Hazen, M. Mathieu, K. McCutcheon, K. Takeda, S. Date, T. K. Cheung, Q. Phung, P. Hass, D. Arnott, J. A. Hongo, D. J. Matthews, A. Brown, A. H. Patel, R. F. Kelley, C. Eigenbrot, and S. B. Kapadia. 2013. 'Glycan shifting on hepatitis C virus (HCV) E2 glycoprotein is a mechanism for escape from broadly neutralizing antibodies', *J Mol Biol*, 425: 1899-914.
- Park, S. H., and B. Rehermann. 2014. 'Immune responses to HCV and other hepatitis viruses', *Immunity*, 40: 13-24.
- Patel, M., P. G. Horgan, D. C. McMillan, and J. Edwards. 2018. 'NF-kappaB pathways in the development and progression of colorectal cancer', *Transl Res*, 197: 43-56.
- Paul, D., S. Hoppe, G. Saher, J. Krijnse-Locker, and R. Bartenschlager. 2013. 'Morphological and biochemical characterization of the membranous hepatitis C virus replication compartment', *J Virol*, 87: 10612-27.
- Paul, D., V. Madan, and R. Bartenschlager. 2014. 'Hepatitis C virus RNA replication and assembly: living on the fat of the land', *Cell Host Microbe*, 16: 569-79.
- Pawlotsky, J. M. 2016. 'Hepatitis C Virus Resistance to Direct-Acting Antiviral Drugs in Interferon-Free Regimens', *Gastroenterology*, 151: 70-86.
- Perez, S., A. Kaspi, T. Domovitz, A. Davidovich, A. Lavi-Itzkovitz, T. Meirson, J. Alison Holmes, C. Y. Dai, C. F. Huang, R. T. Chung, A. Nimer, A. El-Osta, G. Yaari, S. M. Stemmer, M. L. Yu, I. Haviv, and M. Gal-Tanamy. 2019. 'Hepatitis C virus leaves an epigenetic signature post cure of infection by direct-acting antivirals', *PLoS Genet*, 15: e1008181.
- Phan, T., R. K. Beran, C. Peters, I. C. Lorenz, and B. D. Lindenbach. 2009. 'Hepatitis C virus NS2 protein contributes to virus particle assembly via opposing epistatic interactions with the E1-E2 glycoprotein and NS3-NS4A enzyme complexes', *J Virol*, 83: 8379-95.
- Phueaouan, T., P. Chaiyawat, P. Netsirisawan, D. Chokchaichamnankit, P. Punyarit, C. Srisomsap, J. Svasti, and V. Champattanachai. 2013. 'Aberrant O-GlcNAc-modified proteins expressed in primary colorectal cancer', *Oncol Rep*, 30: 2929-36.
- Pietschmann, T., A. Kaul, G. Koutsoudakis, A. Shavinskaya, S. Kallis, E. Steinmann, K. Abid, F. Negro, M. Dreux, F. L. Cosset, and R. Bartenschlager. 2006. 'Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras', *Proc Natl Acad Sci U S A*, 103: 7408-13.
- Pileri, P., Y. Uematsu, S. Campagnoli, G. Galli, F. Falugi, R. Petracca, A. J. Weiner, M. Houghton, D. Rosa, G. Grandi, and S. Abrignani. 1998. 'Binding of hepatitis C virus to CD81', *Science*, 282: 938-41.
- Pinho, T. S., D. M. Verde, S. C. Correia, S. M. Cardoso, and P. I. Moreira. 2018. 'O-GlcNAcylation and neuronal energy status: Implications for Alzheimer's disease', *Ageing Res Rev*, 46: 32-41.
- Piver, Eric, Audrey Boyer, Julien Gaillard, Anne Bull, Elodie Beaumont, Philippe Roingeard, and Jean-Christophe Meunier. 2017. 'Ultrastructural organisation of HCV from the bloodstream of infected patients revealed by electron microscopy after specific immunocapture', *Gut*, 66: 1487-95.
- Ploss, A., M. J. Evans, V. A. Gaysinskaya, M. Panis, H. You, Y. P. de Jong, and C. M. Rice. 2009. 'Human occludin is a hepatitis C virus entry factor required for infection of mouse cells', *Nature*, 457: 882-6.
- Ploss, A., S. R. Khetani, C. T. Jones, A. J. Syder, K. Trehan, V. A. Gaysinskaya, K. Mu, K. Ritola, C. M. Rice, and S. N. Bhatia. 2010. 'Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures', *Proc Natl Acad Sci U S A*, 107: 3141-5.

- Podevin, P., A. Carpentier, V. Pene, L. Aoudjehane, M. Carriere, S. Zaidi, C. Hernandez, V. Calle, J. F. Meritet, O. Scatton, M. Dreux, F. L. Cosset, T. Wakita, R. Bartenschlager, S. Demignot, F. Conti, A. R. Rosenberg, and Y. Calmus. 2010. 'Production of infectious hepatitis C virus in primary cultures of human adult hepatocytes', *Gastroenterology*, 139: 1355-64.
- Popescu, C. I., N. Callens, D. Trinel, P. Roingeard, D. Moradpour, V. Descamps, G. Duverlie, F. Penin, L. Heliot, Y. Rouille, and J. Dubuisson. 2011. 'NS2 protein of hepatitis C virus interacts with structural and non-structural proteins towards virus assembly', *PLoS Pathog*, 7: e1001278.
- Popescu, C. I., L. Riva, O. Vlaicu, R. Farhat, Y. Rouille, and J. Dubuisson. 2014. 'Hepatitis C virus life cycle and lipid metabolism', *Biology (Basel)*, 3: 892-921.
- Reiss, S., I. Rebhan, P. Backes, I. Romero-Brey, H. Erfle, P. Matula, L. Kaderali, M. Poenisch, H. Blankenburg, M. S. Hiet, T. Longerich, S. Diehl, F. Ramirez, T. Balla, K. Rohr, A. Kaul, S. Buhler, R. Pepperkok, T. Lengauer, M. Albrecht, R. Eils, P. Schirmacher, V. Lohmann, and R. Bartenschlager. 2011. 'Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment', *Cell Host Microbe*, 9: 32-45.
- Rozanski, W., A. Krzeslak, E. Forma, M. Brys, M. Blewniewski, P. Wozniak, and M. Lipinski. 2012. 'Prediction of bladder cancer based on urinary content of MGEA5 and OGT mRNA level', *Clin Lab*, 58: 579-83.
- Rumin, S., P. Berthillon, E. Tanaka, K. Kiyosawa, M. A. Trabaud, T. Bizollon, C. Gouillat, P. Gripon, C. Guguen-Guillouzo, G. Inchauspe, and C. Trepo. 1999. 'Dynamic analysis of hepatitis C virus replication and quasispecies selection in long-term cultures of adult human hepatocytes infected in vitro', *J Gen Virol*, 80 (Pt 11): 3007-18.
- Russell, R. S., K. Kawaguchi, J. C. Meunier, S. Takikawa, K. Faulk, J. Bukh, R. H. Purcell, and S. U. Emerson. 2009. 'Mutational analysis of the hepatitis C virus E1 glycoprotein in retroviral pseudoparticles and cell-culture-derived H77/JFH1 chimeric infectious virus particles', *J Viral Hepat*, 16: 621-32.
- Ryerson, A. B., C. R. Ehemann, S. F. Altekruse, J. W. Ward, A. Jemal, R. L. Sherman, S. J. Henley, D. Holtzman, A. Lake, A. M. Noone, R. N. Anderson, J. Ma, K. N. Ly, K. A. Cronin, L. Penberthy, and B. A. Kohler. 2016. 'Annual Report to the Nation on the Status of Cancer, 1975-2012, featuring the increasing incidence of liver cancer', *Cancer*, 122: 1312-37.
- Sainz, B., Jr., and F. V. Chisari. 2006. 'Production of infectious hepatitis C virus by well-differentiated, growth-arrested human hepatoma-derived cells', *J Virol*, 80: 10253-7.
- Saliminejad, K., H. R. Khorram Khorshid, S. Soleymani Fard, and S. H. Ghaffari. 2019. 'An overview of microRNAs: Biology, functions, therapeutics, and analysis methods', *J Cell Physiol*, 234: 5451-65.
- Salloum, S., H. Wang, C. Ferguson, R. G. Parton, and A. W. Tai. 2013. 'Rab18 binds to hepatitis C virus NS5A and promotes interaction between sites of viral replication and lipid droplets', *PLoS Pathog*, 9: e1003513.
- Santolini, E., G. Migliaccio, and N. La Monica. 1994. 'Biosynthesis and biochemical properties of the hepatitis C virus core protein', *J Virol*, 68: 3631-41.
- Santos, C. R., and A. Schulze. 2012. 'Lipid metabolism in cancer', *Febs j*, 279: 2610-23.
- Scarselli, E., H. Ansuini, R. Cerino, R. M. Roccasecca, S. Acali, G. Filocamo, C. Traboni, A. Nicosia, R. Cortese, and A. Vitelli. 2002. 'The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus', *EMBO J*, 21: 5017-25.
- Scheel, T. K., J. M. Gottwein, T. H. Carlsen, Y. P. Li, T. B. Jensen, U. Spengler, N. Weis, and J. Bukh. 2011. 'Efficient culture adaptation of hepatitis C virus recombinants with genotype-specific core-NS2 by using previously identified mutations', *J Virol*, 85: 2891-906.
- Scheel, T. K., J. M. Gottwein, T. B. Jensen, J. C. Prentoe, A. M. Hoegh, H. J. Alter, J. Eugen-Olsen, and J. Bukh. 2008. 'Development of JFH1-based cell culture systems for hepatitis C virus

- genotype 4a and evidence for cross-genotype neutralization', *Proc Natl Acad Sci U S A*, 105: 997-1002.
- Shafi, R., S. P. Iyer, L. G. Ellies, N. O'Donnell, K. W. Marek, D. Chui, G. W. Hart, and J. D. Marth. 2000. 'The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny', *Proc Natl Acad Sci U S A*, 97: 5735-9.
- Shanmugam, S., D. Saravanabalaji, and M. Yi. 2015. 'Detergent-resistant membrane association of NS2 and E2 during hepatitis C virus replication', *J Virol*, 89: 4562-74.
- Sharma, N. R., G. Mateu, M. Dreux, A. Grakoui, F. L. Cosset, and G. B. Melikyan. 2011. 'Hepatitis C virus is primed by CD81 protein for low pH-dependent fusion', *J Biol Chem*, 286: 30361-76.
- Sharma, N. S., V. K. Gupta, P. Dauer, K. Kesh, R. Hadad, B. Giri, A. Chandra, V. Dudeja, C. Slawson, S. Banerjee, S. M. Vickers, A. Saluja, and S. Banerjee. 2019. 'O-GlcNAc modification of Sox2 regulates self-renewal in pancreatic cancer by promoting its stability', *Theranostics*, 9: 3410-24.
- Shavinskaya, A., S. Boulant, F. Penin, J. McLauchlan, and R. Bartenschlager. 2007. 'The lipid droplet binding domain of hepatitis C virus core protein is a major determinant for efficient virus assembly', *J Biol Chem*, 282: 37158-69.
- Shelness, G. S., and J. A. Sellers. 2001. 'Very-low-density lipoprotein assembly and secretion', *Curr Opin Lipidol*, 12: 151-7.
- Shi, Q., J. Jiang, and G. Luo. 2013. 'Syndecan-1 serves as the major receptor for attachment of hepatitis C virus to the surfaces of hepatocytes', *J Virol*, 87: 6866-75.
- Shi, S. T., S. J. Polyak, H. Tu, D. R. Taylor, D. R. Gretch, and M. M. Lai. 2002. 'Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins', *Virology*, 292: 198-210.
- Shi, Y., J. Tomic, F. Wen, S. Shaha, A. Bahlo, R. Harrison, J. W. Dennis, R. Williams, B. J. Gross, S. Walker, J. Zuccolo, J. P. Deans, G. W. Hart, and D. E. Spaner. 2010. 'Aberrant O-GlcNAcylation characterizes chronic lymphocytic leukemia', *Leukemia*, 24: 1588-98.
- Shimakami, T., D. Yamane, R. K. Jangra, B. J. Kempf, C. Spaniel, D. J. Barton, and S. M. Lemon. 2012. 'Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex', *Proc Natl Acad Sci U S A*, 109: 941-6.
- Shimizu, Y., Y. Shirasago, M. Kondoh, T. Suzuki, T. Wakita, K. Hanada, K. Yagi, and M. Fukasawa. 2018. 'Monoclonal Antibodies against Occludin Completely Prevented Hepatitis C Virus Infection in a Mouse Model', *J Virol*, 92.
- Simmonds, P., J. Bukh, C. Combet, G. Deleage, N. Enomoto, S. Feinstone, P. Halfon, G. Inchauspe, C. Kuiken, G. Maertens, M. Mizokami, D. G. Murphy, H. Okamoto, J. M. Pawlotsky, F. Penin, E. Sablon, I. T. Shin, L. J. Stuyver, H. J. Thiel, S. Viazov, A. J. Weiner, and A. Widell. 2005. 'Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes', *Hepatology*, 42: 962-73.
- Smith, D. B., J. Bukh, C. Kuiken, A. S. Muerhoff, C. M. Rice, J. T. Stapleton, and P. Simmonds. 2014. 'Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource', *Hepatology*, 59: 318-27.
- Sodi, V. L., Z. A. Bacigalupa, C. M. Ferrer, J. V. Lee, W. A. Gocal, D. Mukhopadhyay, K. E. Wellen, M. Ivan, and M. J. Reginato. 2018. 'Nutrient sensor O-GlcNAc transferase controls cancer lipid metabolism via SREBP-1 regulation', *Oncogene*, 37: 924-34.
- Sourisseau, M., M. L. Michta, C. Zony, B. Israelow, S. E. Hopcraft, C. M. Narbus, A. Parra Martin, and M. J. Evans. 2013. 'Temporal analysis of hepatitis C virus cell entry with occludin directed blocking antibodies', *PLoS Pathog*, 9: e1003244.
- Stapleford, K. A., and B. D. Lindenbach. 2011. 'Hepatitis C virus NS2 coordinates virus particle assembly through physical interactions with the E1-E2 glycoprotein and NS3-NS4A enzyme complexes', *J Virol*, 85: 1706-17.
- Steinmann, E., and T. Pietschmann. 2013. 'Cell culture systems for hepatitis C virus', *Curr Top Microbiol Immunol*, 369: 17-48.

- Stelma, F., M. H. van der Ree, M. J. Sinnige, A. Brown, L. Swadling, J. M. L. de Vree, S. B. Willemse, M. van der Valk, P. Grint, S. Neben, P. Klenerman, E. Barnes, N. A. Kootstra, and H. W. Reesink. 2017. 'Immune phenotype and function of natural killer and T cells in chronic hepatitis C patients who received a single dose of anti-MicroRNA-122, RG-101', *Hepatology*, 66: 57-68.
- Sulkowski, M. S., D. F. Gardiner, M. Rodriguez-Torres, K. R. Reddy, T. Hassanein, I. Jacobson, E. Lawitz, A. S. Lok, F. Hineostroza, P. J. Thuluvath, H. Schwartz, D. R. Nelson, G. T. Everson, T. Eley, M. Wind-Rotolo, S. P. Huang, M. Gao, D. Hernandez, F. McPhee, D. Sherman, R. Hindes, W. Symonds, C. Pasquinelli, and D. M. Grasela. 2014. 'Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection', *N Engl J Med*, 370: 211-21.
- Sumpter, R., Jr., Y. M. Loo, E. Foy, K. Li, M. Yoneyama, T. Fujita, S. M. Lemon, and M. Gale, Jr. 2005. 'Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I', *J Virol*, 79: 2689-99.
- Swinnen, J. V., K. Brusselmans, and G. Verhoeven. 2006. 'Increased lipogenesis in cancer cells: new players, novel targets', *Curr Opin Clin Nutr Metab Care*, 9: 358-65.
- Syder, A. J., H. Lee, M. B. Zeisel, J. Grove, E. Soulier, J. Macdonald, S. Chow, J. Chang, T. F. Baumert, J. A. McKeating, J. McKelvy, and F. Wong-Staal. 2011. 'Small molecule scavenger receptor BI antagonists are potent HCV entry inhibitors', *J Hepatol*, 54: 48-55.
- Syed, G. H., M. Khan, S. Yang, and A. Siddiqui. 2017. 'Hepatitis C Virus Lipovirions Assemble in the Endoplasmic Reticulum (ER) and Bud off from the ER to the Golgi Compartment in COPII Vesicles', *J Virol*, 91.
- Szymura, S. J., J. P. Zaemes, D. F. Allison, S. H. Clift, J. M. D'Innocenzi, L. G. Gray, B. D. McKenna, B. B. Morris, S. Bekiranov, R. D. LeGallo, D. R. Jones, and M. W. Mayo. 2019. 'NF-kappaB upregulates glutamine-fructose-6-phosphate transaminase 2 to promote migration in non-small cell lung cancer', *Cell Commun Signal*, 17: 24.
- Takacs, C. N., U. Andreo, V. L. Dao Thi, X. Wu, C. E. Gleason, M. S. Itano, G. S. Spitz-Becker, R. L. Belote, B. R. Hedin, M. A. Scull, C. M. Rice, and S. M. Simon. 2017. 'Differential Regulation of Lipoprotein and Hepatitis C Virus Secretion by Rab1b', *Cell Rep*, 21: 431-41.
- Thomssen, R., S. Bonk, C. Propfe, K. H. Heermann, H. G. Kochel, and A. Uy. 1992. 'Association of hepatitis C virus in human sera with beta-lipoprotein', *Med Microbiol Immunol*, 181: 293-300.
- Timpe, J. M., Z. Stamataki, A. Jennings, K. Hu, M. J. Farquhar, H. J. Harris, A. Schwarz, I. Desombere, G. L. Roels, P. Balfe, and J. A. McKeating. 2008. 'Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies', *Hepatology*, 47: 17-24.
- Tomei, L., C. Failla, E. Santolini, R. De Francesco, and N. La Monica. 1993. 'NS3 is a serine protease required for processing of hepatitis C virus polyprotein', *J Virol*, 67: 4017-26.
- Tsukiyama-Kohara, K., N. Iizuka, M. Kohara, and A. Nomoto. 1992. 'Internal ribosome entry site within hepatitis C virus RNA', *J Virol*, 66: 1476-83.
- van der Ree, M. H., J. M. de Vree, F. Stelma, S. Willemse, M. van der Valk, S. Rietdijk, R. Molenkamp, J. Schinkel, A. C. van Nuenen, U. Beuers, S. Hadi, M. Harbers, E. van der Veer, K. Liu, J. Grundy, A. K. Patick, A. Pavlicek, J. Blem, M. Huang, P. Grint, S. Neben, N. W. Gibson, N. A. Kootstra, and H. W. Reesink. 2017. 'Safety, tolerability, and antiviral effect of RG-101 in patients with chronic hepatitis C: a phase 1B, double-blind, randomised controlled trial', *Lancet*, 389: 709-17.
- Vanwolleghem, T., J. Bukh, P. Meuleman, I. Desombere, J. C. Meunier, H. Alter, R. H. Purcell, and G. Leroux-Roels. 2008. 'Polyclonal immunoglobulins from a chronic hepatitis C virus patient protect human liver-chimeric mice from infection with a homologous hepatitis C virus strain', *Hepatology*, 47: 1846-55.
- Vieyres, G., J. Dubuisson, and T. Pietschmann. 2014. 'Incorporation of hepatitis C virus E1 and E2 glycoproteins: the keystones on a peculiar virion', *Viruses*, 6: 1149-87.

- Vieyres, G., and T. Pietschmann. 2013. 'Entry and replication of recombinant hepatitis C viruses in cell culture', *Methods*, 59: 233-48.
- . 2019. 'HCV Pit Stop at the Lipid Droplet: Refuel Lipids and Put on a Lipoprotein Coat before Exit', *Cells*, 8.
- Vieyres, G., X. Thomas, V. Descamps, G. Duverlie, A. H. Patel, and J. Dubuisson. 2010. 'Characterization of the envelope glycoproteins associated with infectious hepatitis C virus', *J Virol*, 84: 10159-68.
- Wakita, T., T. Pietschmann, T. Kato, T. Date, M. Miyamoto, Z. Zhao, K. Murthy, A. Habermann, H. G. Krausslich, M. Mizokami, R. Bartenschlager, and T. J. Liang. 2005. 'Production of infectious hepatitis C virus in tissue culture from a cloned viral genome', *Nat Med*, 11: 791-6.
- Walker, C. M. 1997. 'Comparative features of hepatitis C virus infection in humans and chimpanzees', *Springer Semin Immunopathol*, 19: 85-98.
- Wang, C., P. Sarnow, and A. Siddiqui. 1993. 'Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism', *J Virol*, 67: 3338-44.
- Wang, L., S. Chen, Z. Zhang, J. Zhang, S. Mao, J. Zheng, Y. Xuan, M. Liu, K. Cai, W. Zhang, Y. Guo, W. Zhai, and X. Yao. 2018. 'Suppressed OGT expression inhibits cell proliferation while inducing cell apoptosis in bladder cancer', *BMC Cancer*, 18: 1141.
- Warburg, O. 1956. 'On respiratory impairment in cancer cells', *Science*, 124: 269-70.
- Wasilewski, L. N., S. C. Ray, and J. R. Bailey. 2016. 'Hepatitis C virus resistance to broadly neutralizing antibodies measured using replication-competent virus and pseudoparticles', *J Gen Virol*, 97: 2883-93.
- Wernette-Hammond, M. E., S. J. Lauer, A. Corsini, D. Walker, J. M. Taylor, and S. C. Rall, Jr. 1989. 'Glycosylation of human apolipoprotein E. The carbohydrate attachment site is threonine 194', *J Biol Chem*, 264: 9094-101.
- Wieland, S., Z. Makowska, B. Campana, D. Calabrese, M. T. Dill, J. Chung, F. V. Chisari, and M. H. Heim. 2014. 'Simultaneous detection of hepatitis C virus and interferon stimulated gene expression in infected human liver', *Hepatology*, 59: 2121-30.
- Witteveldt, J., M. J. Evans, J. Bitzegeio, G. Koutsoudakis, A. M. Owsianka, A. G. Angus, Z. Y. Keck, S. K. Fong, T. Pietschmann, C. M. Rice, and A. H. Patel. 2009. 'CD81 is dispensable for hepatitis C virus cell-to-cell transmission in hepatoma cells', *J Gen Virol*, 90: 48-58.
- Wong, L., and A. Torbati. 1994. 'Differentiation of intrahepatic membrane-bound and secretory apolipoprotein B by monoclonal antibodies: membrane-bound apolipoprotein B is more glycosylated', *Biochemistry*, 33: 1923-9.
- Wensch, F., E. Crouchet, G. Ligat, M. B. Zeisel, Z. Y. Keck, S. K. H. Fong, C. Schuster, and T. F. Baumert. 2018. 'Hepatitis C Virus (HCV)-Apolipoprotein Interactions and Immune Evasion and Their Impact on HCV Vaccine Design', *Front Immunol*, 9: 1436.
- Xiao, F., I. Fofana, C. Thumann, L. Mailly, R. Alles, E. Robinet, N. Meyer, M. Schaeffer, F. Habersetzer, M. Doffoel, P. Leyssen, J. Neyts, M. B. Zeisel, and T. F. Baumert. 2015. 'Synergy of entry inhibitors with direct-acting antivirals uncovers novel combinations for prevention and treatment of hepatitis C', *Gut*, 64: 483-94.
- Xie, Z. C., J. I. Riezu-Boj, J. J. Lasarte, J. Guillen, J. H. Su, M. P. Civeira, and J. Prieto. 1998. 'Transmission of hepatitis C virus infection to tree shrews', *Virology*, 244: 513-20.
- Xu, W., X. Zhang, J. L. Wu, L. Fu, K. Liu, D. Liu, G. G. Chen, P. B. Lai, N. Wong, and J. Yu. 2017. 'O-GlcNAc transferase promotes fatty liver-associated liver cancer through inducing palmitic acid and activating endoplasmic reticulum stress', *J Hepatol*, 67: 310-20.
- Xu, X., H. Chen, X. Cao, and K. Ben. 2007. 'Efficient infection of tree shrew (*Tupaia belangeri*) with hepatitis C virus grown in cell culture or from patient plasma', *J Gen Virol*, 88: 2504-12.
- Xu, Y., P. Martinez, K. Seron, G. Luo, F. Allain, J. Dubuisson, and S. Belouzard. 2015. 'Characterization of hepatitis C virus interaction with heparan sulfate proteoglycans', *J Virol*, 89: 3846-58.

- Yang, W. H., S. Y. Park, H. W. Nam, D. H. Kim, J. G. Kang, E. S. Kang, Y. S. Kim, H. C. Lee, K. S. Kim, and J. W. Cho. 2008. 'NFkappaB activation is associated with its O-GlcNAcylation state under hyperglycemic conditions', *Proc Natl Acad Sci U S A*, 105: 17345-50.
- Yang, X., and K. Qian. 2017. 'Protein O-GlcNAcylation: emerging mechanisms and functions', *Nat Rev Mol Cell Biol*, 18: 452-65.
- Yang, Y. R., M. Song, H. Lee, Y. Jeon, E. J. Choi, H. J. Jang, H. Y. Moon, H. Y. Byun, E. K. Kim, D. H. Kim, M. N. Lee, A. Koh, J. Ghim, J. H. Choi, W. Lee-Kwon, K. T. Kim, S. H. Ryu, and P. G. Suh. 2012. 'O-GlcNAcase is essential for embryonic development and maintenance of genomic stability', *Aging Cell*, 11: 439-48.
- Yehezkel, G., L. Cohen, A. Kliger, E. Manor, and I. Khalaila. 2012. 'O-linked beta-N-acetylglucosaminylation (O-GlcNAcylation) in primary and metastatic colorectal cancer clones and effect of N-acetyl-beta-D-glucosaminidase silencing on cell phenotype and transcriptome', *J Biol Chem*, 287: 28755-69.
- Yi, M., Y. Ma, J. Yates, and S. M. Lemon. 2009. 'Trans-complementation of an NS2 defect in a late step in hepatitis C virus (HCV) particle assembly and maturation', *PLoS Pathog*, 5: e1000403.
- Yuzwa, S. A., M. S. Macauley, J. E. Heinonen, X. Shan, R. J. Dennis, Y. He, G. E. Whitworth, K. A. Stubbs, E. J. McEachern, G. J. Davies, and D. J. Vocadlo. 2008. 'A potent mechanism-inspired O-GlcNAcase inhibitor that blocks phosphorylation of tau in vivo', *Nat Chem Biol*, 4: 483-90.
- Yuzwa, S. A., X. Shan, M. S. Macauley, T. Clark, Y. Skorobogatko, K. Vosseller, and D. J. Vocadlo. 2012. 'Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation', *Nat Chem Biol*, 8: 393-9.
- Zachara, N., Y. Akimoto, and G. W. Hart. 2015. 'The O-GlcNAc Modification.' in rd, A. Varki, R. D. Cummings, J. D. Esko, P. Stanley, G. W. Hart, M. Aebi, A. G. Darvill, T. Kinoshita, N. H. Packer, J. H. Prestegard, R. L. Schnaar and P. H. Seeberger (eds.), *Essentials of Glycobiology* (Cold Spring Harbor Laboratory Press
- Copyright 2015-2017 by The Consortium of Glycobiology Editors, La Jolla, California. All rights reserved.: Cold Spring Harbor (NY)).
- Zahid, M. N., M. Turek, F. Xiao, V. L. Thi, M. Guerin, I. Fofana, P. Bachellier, J. Thompson, L. Delang, J. Neyts, D. Bankwitz, T. Pietschmann, M. Dreux, F. L. Cosset, F. Grunert, T. F. Baumert, and M. B. Zeisel. 2013. 'The postbinding activity of scavenger receptor class B type I mediates initiation of hepatitis C virus infection and viral dissemination', *Hepatology*, 57: 492-504.
- Zeisel, M. B., F. L. Cosset, and T. F. Baumert. 2008. 'Host neutralizing responses and pathogenesis of hepatitis C virus infection', *Hepatology*, 48: 299-307.
- Zeisel, M. B., E. Crouchet, T. F. Baumert, and C. Schuster. 2015. 'Host-Targeting Agents to Prevent and Cure Hepatitis C Virus Infection', *Viruses*, 7: 5659-85.
- Zeisel, M. B., P. Dhawan, and T. F. Baumert. 2018. 'Tight junction proteins in gastrointestinal and liver disease', *Gut*.
- Zeisel, M. B., D. J. Felmlee, and T. F. Baumert. 2013. 'Hepatitis C virus entry', *Curr Top Microbiol Immunol*, 369: 87-112.
- Zeisel, M. B., J. Lupberger, I. Fofana, and T. F. Baumert. 2013. 'Host-targeting agents for prevention and treatment of chronic hepatitis C - perspectives and challenges', *J Hepatol*, 58: 375-84.
- Zeisel, Mirjam B., and Thomas F. Baumert. 2017. 'Clinical development of hepatitis C virus host-targeting agents', *The Lancet*, 389: 674-75.
- Zeng, Q., R. X. Zhao, J. Chen, Y. Li, X. D. Li, X. L. Liu, W. M. Zhang, C. S. Quan, Y. S. Wang, Y. X. Zhai, J. W. Wang, M. Youssef, R. Cui, J. Liang, N. Genovese, L. T. Chow, Y. L. Li, and Z. X. Xu. 2016. 'O-linked GlcNAcylation elevated by HPV E6 mediates viral oncogenesis', *Proc Natl Acad Sci U S A*, 113: 9333-8.

- Zeuzem, S., P. Andreone, S. Pol, E. Lawitz, M. Diago, S. Roberts, R. Focaccia, Z. Younossi, G. R. Foster, A. Horban, P. Ferenci, F. Nevens, B. Mullhaupt, P. Pockros, R. Terg, D. Shouval, B. van Hoek, O. Weiland, R. Van Heeswijk, S. De Meyer, D. Luo, G. Boogaerts, R. Polo, G. Picchio, and M. Beumont. 2011. 'Telaprevir for retreatment of HCV infection', *N Engl J Med*, 364: 2417-28.
- Zhang, P., C. Wang, T. Ma, and S. You. 2015. 'O-GlcNAcylation enhances the invasion of thyroid anaplastic cancer cells partially by PI3K/Akt1 pathway', *Oncotargets Ther*, 8: 3305-13.
- Zhang, X., T. Wang, X. Dai, Y. Zhang, H. Jiang, Q. Zhang, F. Liu, K. Wu, Y. Liu, H. Zhou, and J. Wu. 2016. 'Golgi protein 73 facilitates the interaction of hepatitis C virus NS5A with apolipoprotein E to promote viral particle secretion', *Biochem Biophys Res Commun*, 479: 683-89.
- Zhong, J., P. Gastaminza, G. Cheng, S. Kapadia, T. Kato, D. R. Burton, S. F. Wieland, S. L. Uprichard, T. Wakita, and F. V. Chisari. 2005. 'Robust hepatitis C virus infection in vitro', *Proc Natl Acad Sci U S A*, 102: 9294-9.
- Zhu, Q., L. Zhou, Z. Yang, M. Lai, H. Xie, L. Wu, C. Xing, F. Zhang, and S. Zheng. 2012. 'O-GlcNAcylation plays a role in tumor recurrence of hepatocellular carcinoma following liver transplantation', *Med Oncol*, 29: 985-93.
- Zhu, Y., T. W. Liu, Z. Madden, S. A. Yuzwa, K. Murray, S. Cecioni, N. Zachara, and D. J. Vocadlo. 2016. 'Post-translational O-GlcNAcylation is essential for nuclear pore integrity and maintenance of the pore selectivity filter', *J Mol Cell Biol*, 8: 2-16.
- Zona, L., J. Lupberger, N. Sidahmed-Adrar, C. Thumann, H. J. Harris, A. Barnes, J. Florentin, R. G. Tawar, F. Xiao, M. Turek, S. C. Durand, F. H. Duong, M. H. Heim, F. L. Cosset, I. Hirsch, D. Samuel, L. Brino, M. B. Zeisel, F. Le Naour, J. A. McKeating, and T. F. Baumert. 2013. 'HRas signal transduction promotes hepatitis C virus cell entry by triggering assembly of the host tetraspanin receptor complex', *Cell Host Microbe*, 13: 302-13.
- Zucman-Rossi, J., A. Villanueva, J. C. Nault, and J. M. Llovet. 2015. 'Genetic Landscape and Biomarkers of Hepatocellular Carcinoma', *Gastroenterology*, 149: 1226-39 e4.

VIII. ANNEX

Review: Plissonnier ML, **Herzog K**, Levrero M, Zeisel MB. Non-coding RNA and HCV-induced HCC.
Viruses 2018, 10 (11): 591

Review

Non-Coding RNAs and Hepatitis C Virus-Induced Hepatocellular Carcinoma

Marie-Laure Plissonnier ¹, Katharina Herzog ^{2,3}, Massimo Levrero ^{1,4} and Mirjam B. Zeisel ^{1,*}

¹ Cancer Research Center of Lyon (CRCL), UMR Inserm 1052 CNRS 5286 Mixte CLB, Université de Lyon 1 (UCBL1), 69003 Lyon, France; marie-laure.plissonnier@inserm.fr (M.-L.P.); massimo.levrero@inserm.fr (M.L.)

² Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, 67000 Strasbourg, France; katharina.herzog@etu.unistra.fr

³ Université de Strasbourg, 67000 Strasbourg, France

⁴ Hospices Civils de Lyon, Service d'Hépatogastroentérologie, 69004 Lyon, France

* Correspondence: mirjam.zeisel@inserm.fr; Tel.: +33472681970

Received: 16 October 2018; Accepted: 29 October 2018; Published: 30 October 2018



Abstract: Hepatitis C virus (HCV) infection is a worldwide health problem and is one of the main causes of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Despite recent improvements, effective treatments for HCC are still missing and new tools for early detection are needed. Non-coding RNAs (ncRNAs) have emerged as important regulators of gene expression and key players in human carcinogenesis, including HCC. Aberrant expression of ncRNAs is associated with HCC metastasis, invasion, dissemination, and recurrence. This review will focus on the recent advances in ncRNA expression profiles, their dysregulation in HCV-related HCC, and the clinical perspective of ncRNA signatures for the early detection of HCC.

Keywords: hepatocellular carcinoma; hepatitis C virus; microRNA; long non-coding RNA

1. Introduction

Despite an overall drop in the incidence of hepatitis C virus (HCV) infection within the past years due to direct-acting antivirals (DAAs), which enable chronic hepatitis C (CHC) to be cured, an estimated 71 million individuals are still chronically infected by HCV worldwide [1]. HCV infection is a major risk factor of HCC and associated with 20% of cases of liver cirrhosis, which is functional decompensation leading to hepatocellular carcinoma (HCC) [2]. Other major HCC etiologies include chronic hepatitis B virus (HBV) infection, alcohol abuse, and metabolic causes. In HCV-infected patients, HCC development is a consequence of fibrosis progression and occurs after the establishment of cirrhosis [3]. According to the GLOBOCAN series of the International Agency for Research on Cancer in 2012 [4], HCC is the fifth most common cancer and the second cause of cancer death worldwide.

HCC is a poor prognosis disease. Although the risk of developing HCC can be reduced in patients by treatment of the underlying cause, e.g., by viral clearance or abstinence from alcohol, strategies to prevent cancer development in patients with advanced fibrosis and/or established cirrhosis are still lacking. Early diagnosis increases the chance of effective therapy, but the detection of small liver tumours by ultrasound is challenging and currently there is no reliable serum biomarker that can be used in surveillance programmes. Patients with advanced HCC carry a very poor prognosis, with an expected survival of four to six months and despite recent improvements, treatment options for HCC remain scarce. According to the clinical practice guidelines from the European Association for the Study of the liver (EASL), patients eligible for curative treatment can undergo surgical resection, radiofrequency ablation, or liver transplantation [5]. Tumour recurrence remains the major cause of

death in HCC patients following loco-regional ablation and liver resection. Intermediate (stage B according to the Barcelona–Clínica Liver Cancer (BCLC) classification [5]) HCCs have been shown to benefit from trans-arterial chemoembolization (TACE) [5]. The first-line treatments for patients with advanced HCC (BCLC stage C) [5], which are not eligible for curative treatment, are the multikinase inhibitors sorafenib and lenvatinib, which increase survival by approximately three months [6]. Phase III clinical trials with regorafenib or cabozantinib as second-line treatment for HCC patients undergoing tumour progression after sorafenib (RESORCE, CELESTIAL) showed an extension of overall survival by 10 months [7,8]. Novel treatment options and prognostic tools are required to improve the management of patients with HCC.

Among the risk factors of HCC, HCV is a positive-strand RNA virus [9,10] that does not integrate into the host genome. It encodes a large polyprotein of about 3000 amino acids from a single open reading frame which is processed into three structural (core, E1, and E2) and seven non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [11]. HCV proteins interact with many host-cell factors well beyond their roles in the viral life cycle and are notably involved in cell signalling, transcription, cell proliferation, apoptosis, vesicular trafficking, and translational regulation [12]. HCV proteins contribute to HCC by modulating pathways that promote the malignant transformation of hepatocytes through the accumulation of genetic damages and epigenetic dysregulation [13–15]. For instance, the HCV core protein sensitizes host cells to TRAIL-induced cell apoptosis by activating the CK1 α -p53-Bid dependent pathway in human hepatoma cells [16] or is able to suppress the p53-dependent apoptosis induced by the all-*trans* retinoic acid (ATRA), the most biologically active metabolite of vitamin A [17]. Moreover, the HCV core protein binds to several tumour suppressor proteins, including p53, p73, and pRb [18,19]. Despite the development of DAAs enabling an HCV cure and reducing the risk of liver complications [20,21], a long-term risk of HCC remains in cirrhotic patients, even after achieving a sustained virological response (SVR) [22]. Of note, while some reports published within the past two years have raised concerns about a potential higher risk of post-SVR HCC in patients treated with DAAs [23–25] compared to patients treated with the previous standard-of-care interferon and ribavirin, several subsequent reports—including large multicenter studies—have failed to confirm this [21,26–33].

Liver carcinogenesis is a multistep process driven by chronic inflammation, DNA damage, senescence and telomerase reactivation, chromosomal instability, and epigenetic modifications. All etiologic factors seem to act through similar mechanisms (i.e., point mutations, chromosomal aberrations, epigenetic changes) that converge to affect common pathways. Notably, mutations and chromosomal aberrations have been predominantly found in malignant tumour tissues, whereas the dysregulation of signalling pathways and epigenetic changes are also detected earlier in the natural history of HCC development, at the stage of cirrhosis [34]. Epigenetic changes that include DNA methylation, post-translational histone modifications, and ncRNA-mediated silencing pathways occur early in the development of HCC. Several studies have also identified mutations in a group of chromatin regulators (*ARID1A*, *ARID1B*, *ARID2*, *MLL*, and *MLL3*) in approximately 20% of all tumours, including virus- and alcohol-related HCCs (reviewed in [34]). Modulation of the methylation status of DNA CpG islands and lysines in the histone tails in gene promoters represents important epigenetic alterations in human cancer, including HCC. Next-generation sequencing technology has revealed that many ncRNAs play important roles in biological processes, such as differentiation, proliferation, and cell death [35–37], as well as in cancer [38,39]. Based on their length, ncRNAs are classified into small ncRNAs (sncRNAs, less than 200 nucleotides) and long ncRNAs (lncRNAs, more than 200 nucleotides), according to the RNA purification protocol that excludes small RNAs described by Kapranov and colleagues [40]. Aberrant expression of ncRNAs is associated with HCC metastasis, invasion, dissemination, and recurrence [41–45], suggesting that ncRNAs are key players of human carcinogenesis, including HCC. This review will focus on sncRNAs and lncRNAs in HCV-induced HCC.

2. Expression and Functions of ncRNAs in HCV-Related HCC

2.1. LncRNAs in HCV-Related HCC

LncRNAs are a heterogeneous group of non-coding transcripts more than 200 nucleotides long that are transcribed by RNA polymerase II, 5' capped, spliced, and polyadenylated [46,47]. The FANTOM project [48] and the GENCODE consortium in the framework of the ENCODE project [49] led to the identification of over 20,000 lncRNAs. In 2015, an analysis of 7256 RNA-seq libraries from tumours, normal tissues, and cell lines reported the identification of 58,648 lncRNA genes [46].

LncRNAs are involved in many biological processes, such as proliferation, differentiation and development [47,50]. The dysregulation of lncRNAs significantly contributes to numerous human diseases, especially cancers [51,52], including HCC [47,53]. The structural complexity of lncRNAs offers multiple possibilities for interactions with DNA, RNA, and/or proteins that depend on secondary and tertiary structures. Several lncRNAs are localized to specific cellular compartments, nuclear or cytosolic, in accordance with their biological function. Nuclear lncRNAs can act as guides for chromatin-modifying-complexes or transcription factors. The majority of lncRNAs are in the cytoplasm and often function as regulators of protein levels, either by directly controlling mRNA stability or by acting as competing endogenous RNA [54]. Depending on their genomic location and context, lncRNAs are classified into five categories [50,55]: (1) intergenic lncRNAs, the so-called lincRNAs are transcribed in the intergenic region between two protein-coding genes; (2) sense/intronic lncRNAs, which arise from intronic regions within protein-coding genes; (3) antisense lncRNAs (NATs), when overlapping one or more exons of another transcript on the opposite strand; (4) bidirectional lncRNAs, such as promoter upstream transcripts (PROMPTSs), which are transcribed in the promoter regions of protein-coding genes in a bidirectional manner; and (5) enhancer lncRNAs which are transcribed in the enhancer regions of the genome. Two other ncRNAs can be considered as lncRNAs: circular RNAs (circRNAs), which are created from protein-coding mRNAs or linear ncRNAs that join an upstream 3' splice site and downstream 5' splice site to form a covalently closed continuous loop [56]; and pseudogenes, which originate from gene duplication and have acquired various mutations, inducing a loss of their protein-coding capacity [57,58]. Most of these pseudogenes serve as competitive endogenous RNAs (ceRNA) to sequester miRNAs. The *PTEN* pseudogene, *PTENP1*, was the first pseudogene shown to regulate the expression of its parental gene by binding and sequestering *PTEN*-targeting miR-17, miR-19, miR-20a, and miR-21 in prostate cancer [57].

LncRNAs regulate gene expression through different mechanisms, such as epigenetic silencing, splicing regulation, lncRNA-miRNA interaction by sequestering miRNAs, lncRNA-protein interaction, and genetic variation [53,59,60]. The molecular functions of lncRNAs are not well-characterized, but four categories of mode of action have been proposed [39,53]. LncRNAs can function as decoys by binding and titrating away proteins or RNA targets such as miRNAs. This family of lncRNAs can negatively regulate the expression of their target or bind to the transcription binding sites and avoid the fixation of the transcription factor. LncRNAs can also have a guide function: these lncRNAs bind proteins and direct their localization to a specific target. They act, for example, as epigenetic repressors, i.e., HOX transcript antisense intergenic RNA (HOTAIR), or epigenetic activators, such as HOTTIP or H19. Moreover, signal lncRNAs are expressed in a cell-type specific and stage-specific manner and can regulate transcriptional activity or biological pathways by interacting with transcription factors or chromatin-modifying enzymes. Finally, lncRNAs can have a scaffold function. This class of lncRNAs serves as a central platform to bind different molecular components and facilitates their intermolecular interactions.

Many studies have described the role of lncRNAs in HCC (for review see [47,53,61–63]). HCC-related lncRNAs participate in diverse biological processes involved in HCC progression, such as cell proliferation, apoptosis, invasion, metastasis, and angiogenesis. In the last decade, hundreds of dysregulated lncRNAs have been characterized in HCC tissues compared with normal tissues [64]. For example, the lncRNA HOTAIR is highly expressed in HCC and is associated with

poor prognosis [65] and an increased risk of recurrence and metastasis [66,67]. Recently, lncRNA HULC polymorphisms have been associated with HCC risk and prognosis [68]. Furthermore, Guo and colleagues demonstrated that the lncRNA PVT1 is upregulated in HCC, promoting HCC cell propagation and inhibiting apoptotic cells by recruiting EZH2 [69].

lncRNAs can be differentially expressed depending on the HCC etiology. In 2015, Zhang et al. explored lncRNA expression profiles of 73 tissue samples at different stages of HCV-induced HCC: cirrhotic tissue, dysplastic nodules, and HCC samples compared to healthy liver tissue [70]. The expression of seven lncRNAs (LINC01419, BC014579, AK021443, RP11-401P9.4, RP11-304 L19.5, AF070632, CTB-167B5.2) in preneoplastic lesions and HCC was significantly different. Among these lncRNAs, the lncRNA LINC01419 transcripts were expressed at higher levels in early stage HCC compared to dysplasia and early stage HCC, and were overexpressed in HCV-related HCC when compared with matched non-tumour liver tissues. lncRNA AK021443 levels increase in advanced stage HCC, while lncRNA AF070632 levels decrease in advanced stage HCC. Moreover, computational analysis suggested that LINC01419 and AK021443 regulate cell cycle genes, whereas AF070632 is associated with cofactor binding, oxidation-reduction, and the carboxylic acid catabolic process. LINC01419 and AK021443 could thus promote HCV-related HCC development by modulating the cell cycle progression. In another study conducted by Zhang et al. in 2016 [71], lncRNA hypoxia-inducing factor a (aHIF), Prader Willi/Angelman region RNA 5 (PAR5), and human downregulated expression by HBx (hDREH) were associated with HCV-related HCC since their expressions were significantly downregulated (aHIF and PAR5) or upregulated (hDREH) in tumour vs. non-tumour tissues, but these observations have to be confirmed in a larger patient cohort. Two additional lncRNAs, urothelial carcinoma associated-1 (UCA1) and WD repeat containing antisense to TP53 (WRAP53), have been found to be upregulated in HCC patients with chronic HCV infection [72]. UCA1 upregulation has been shown to increase epithelial-to-mesenchymal transition in HCC via sponging miR-203, thereby activating the expression of transcription factor Snail2 [73] (Table 1).

Table 1. Role of lncRNAs in HCV-induced HCC. The expression of lncRNAs that have been associated with HCV-induced HCC, as well as their biological function, are shown. lncRNAs that have been reported to be uniquely modulated in HCC induced by HCV, but not in HCC induced by another etiological factor, are highlighted in bold.

lncRNA	Expression in HCV-Induced HCC	Molecular Mechanism for HCV-Induced HCC	References
HOTAIR	↑	Epigenetic repression	[66,67]
HULC	↑	Polymorphism	[68]
PVT1	↑	Cell cycle progression	[69]
LINC01419	↑	Regulation of cell cycle genes	[70]
BC014579	↑	unknown	[70]
AK021443	↑	Regulation of cell cycle genes	[70]
RP11-401P9.4	↑	unknown	[70]
RP11-304 L19.5	↑	unknown	[70]
AF070632	↓	Cofactor binding and catabolic processes	[70]
CTB-167B5.2	↓	unknown	[70]
aHIF	↓	unknown	[71]
PAR5	↓	unknown	[71]
LINC01152	↓	unknown	[71]
TMEVPG1	↓	unknown	[71]
BC017743	↑	unknown	[71]
BC043430	↑	unknown	[71]
PCNA-AS1	↑	unknown	[71]
UFC1	↑	unknown	[71]
ZEB1-AS1	↑	unknown	[71]
hDREH	↑	unknown	[71]
UCA1	↑	Control of gene expression (target: miR-203)	[72]
WRAP53	↑	unknown	[72]
MALAT1	↑	Regulation of splicing processes	[73]
HEIH	↑	Cell proliferation	[74]

2.2. SncRNA in HCV-Related HCC

High-throughput sequencing technologies have enabled researchers to uncover different types of sncRNA: small nucleolar RNAs (snoRNAs), piwi-interacting RNAs (piRNAs), and miRNAs. Within the last decade, the role of sncRNAs—and particularly the one of miRNAs—in physiological and pathological processes, including HCC, has been extensively studied [38].

2.2.1. Small Nucleolar RNAs (snoRNAs)

SnoRNAs are a class of intermediate-sized ncRNAs of 60 to 300 nucleotides discovered in the nucleolus and able to regulate ribosome maturation and function [75], as well as alternative splicing and gene silencing [76,77]. SnoRNAs are divided into two major highly conserved families based on their structure and main function: box C/D snoRNAs and box H/ACA snoRNAs. Box C/D snoRNAs are responsible for 2'-O-methylation of ribosomal RNAs [78], while the second family of snoRNAs guides the pseudouridylation of nucleotides [79]. A third less represented class includes the small Cajal body-specific RNAs (scaRNAs) that are associated with Cajal bodies, which are small membrane-less sub-compartments of the nucleus [80]. SnoRNAs are located in introns. They are components of small nucleolar ribonucleoprotein (snoRNPs) complexes along with specific proteins and function as a guide for the post-transcriptional modification of ribosomal RNAs by facilitating rRNA folding and stability [81,82]. The sequences of snoRNAs are responsible for targeting the assembled snoRNPs to a specific target.

Alterations in snoRNA expression in human cells can affect numerous biological processes, leading to tumorigenesis. Six snoRNAs are well-described to be dysregulated in HCC [83], regardless of the etiological factor. For example, SNORD113-1 is significantly downregulated in HCC-tumour tissues compared with non-tumour tissues; furthermore, a statistically significant association between low-level expression of SNORD113-1 and relapse-free survival was observed, which suggests that downregulation of SNORD113-1 is associated with HCC aggressiveness [84]. Furthermore, a study performed by Fang et al. [85] demonstrated that SNORD126—located within the intron of the *cyclin B1-interacting protein 1 (CCNB1IP1)* gene—was upregulated to a high level in HCC compared with non-tumour tissues. Overexpression of SNORD126 was associated with a shorter survival rate in HCC patients and promoted HCC growth through upregulation of the PI3K-AKT pathway.

2.2.2. Piwi-Interacting RNAs (piRNAs)

PiRNAs are ncRNAs of 24–30 nucleotides in length that bind to the piwi subfamily of argonaute proteins to form a piRNA-induced silencing complex (piRISC), which inhibits transposon mobilization by both epigenetic and post-transcriptional silencing [86,87]. They are transcribed from regions in the genome that contain transcribed transposable elements and other repetitive elements. Three major PIWI-class proteins (PIWIL1, PIWIL2, and PIWIL4) are involved in a so-called 'ping-pong' amplification cycle, creating antisense piRNAs that are capable of repressing the transcript of origin [87].

PiRNAs are abundant in the human liver, but no data is available on specific piRNA expression profiles in HCV-related HCC. However, it has been shown by small RNA-seq that an expression pattern of 125 piRNAs clearly differentiates cirrhotic liver from HCC tissues. Interestingly, 24 piRNAs dysregulated in advanced HCC also showed distinctive expression patterns in earlier hepatic lesions, suggesting that these ncRNAs may participate in the carcinogenic process in the liver and could represent new markers of early hepatocarcinogenic lesions [87,88]. The accumulation of piR-LLi-30552 and has-piR-020498 is associated with progression from the dysplasia stage to HCC [88]. Furthermore, Law et al. showed that piR-Hep1 is upregulated in 46.6% of HCC tumours compared to the corresponding adjacent non-tumour liver. piR-Hep1 could play a functional role in hepatocarcinogenesis as it has been shown to promote cell viability, motility, and invasiveness, with a concomitant increase in the level of active AKT phosphorylation [89].

2.2.3. MicroRNAs (miRNAs)

MiRNAs are an important class of ncRNAs of 18–25 nucleotides that regulate gene expression at the post-transcriptional level. MiRNAs bind to the 3' untranslated region (3'UTR) of complementary sequences of mRNAs to mediate mRNA deadenylation or translation blockage [90]. MiRNAs are estimated to regulate the translation of more than 60% of protein-coding genes: a single miRNA can target hundreds of mRNAs, thereby affecting a broad network of genes [91].

Biogenesis of miRNAs takes place through a multistep process [92]. miRNAs are most commonly transcribed in the nucleus by the RNA polymerase II (Pol II). Monocistronic or polycistronic primary miRNA transcripts (pri-miRNAs) are processed into precursor miRNAs (pre-miRNAs) by the DGCR8-Drosha complex and exported to the cytoplasm by exportin 5. These pre-miRNAs undergo cleavage by the endoribonuclease called Dicer that produces a miRNA duplex. These molecules are loaded by the Dicer–TARBP2 (TAR RNA-binding protein 2; also known as TRBP) complex into a member of the argonaute protein subfamily to form the RNA-induced silencing complex (RISC), of which argonaute proteins are the catalytic endonuclease components. RISC directs the regulation of mRNAs by recognizing a complementary sequence in the targeted mRNAs. Translation of mRNAs into proteins is repressed by miRNAs by two main means: mRNA degradation and the inhibition of translation initiation [93].

Several studies have investigated the role of miRNAs in various biological processes [38,39,94–96], including proliferation, differentiation, angiogenesis, apoptosis, and development. Abnormal expression levels of miRNAs have been described in inflammation, Alzheimer's disease, cardiovascular disease, cancer, and viral infection, including HCV infection [38,39,42,54,97]. Of note, several studies revealed an association between the dysregulation of miRNAs and HCC carcinogenesis, including HCV-related HCC [42,98,99]. For example, miR-221 that modulates different pathways involved in the proliferation of tumour cells, survival, and metastasis [100,101] is frequently upregulated in HCC with advanced tumour stages and associated with poor prognosis, irrespective of the HCC etiology. In contrast, other miRNAs have been shown to be specifically deregulated in HCV-induced HCC. Using a microarray analysis of liver tissue samples, Diaz et al. identified 18 miRNAs specifically expressed in HCV-related HCC, including 15 miRNAs that had already been reported in previous studies and three miRNAs (miR-497, miR-1269, and miR-424-3p) which had not been previously described to be modulated in HCV-related HCC (Table 2) [42].

Table 2. miRNA-specific signature of HCV-induced HCC. The expression of miRNAs that have been associated with HCV-induced HCC [42], as well as their biological function, are shown.

miRNA	Expression in HCV-Induced HCC	Molecular Mechanism for HCV-Induced HCC
mir-1269	↑	Increase of proliferation
mir-224	↑	Increase of proliferation
mir-452	↑	Increase of proliferation, migration and invasion
mir-224-3p	↑	unknown
mir-224-5p	↑	unknown
mir-221	↑	Increase of proliferation and invasion
mir-497	↓	Inhibition of proliferation, induction of apoptosis
mir-214	↓	Inhibition of proliferation, migration and invasion
mir-195	↓	Inhibition of proliferation and EMT
mir-130a	↓	Inhibition of proliferation, migration and invasion
mir-125a-5p	↓	Inhibition of proliferation
mir-125b-5p	↓	Inhibition of proliferation
mir-424-3p	↓	unknown
mir-139-3p	↓	Inhibition of proliferation and metastasis
mir-139-5p	↓	Inhibition of EMT, migration and invasion
mir-199b-3p	↓	unknown
mir-199a-3p	↓	Inhibition of proliferation, migration, invasion and angiogenesis
mir-199a-5p	↓	Inhibition of proliferation, migration and invasion

Furthermore, it has been shown that the expression of oncogenic miR-155 is increased in patients infected with HCV and this promotes hepatocyte proliferation and tumorigenesis by activating the Wnt signalling pathway [102]. Likewise, miR-135a-5p was shown to be upregulated in HCV-infected patients and to promote the HCV-induced STAT3 transcriptional program in the liver of patients by suppressing its regulator protein tyrosine phosphatase receptor delta (PTPRD), resulting in the malignant progression of liver disease [103]. These studies underscore the functional role that miRNAs may play in the pathogenesis of HCV-induced HCC.

The study of the role of miRNAs in HCV-induced HCC is particularly interesting since there is a tight interplay between HCV, miRNAs, and hepatocyte metabolic pathways that contributes to liver disease development. One of the hallmarks of HCV replication is its dependency on miR-122, the most abundant miRNA in the liver. miR-122 plays a major role in liver physiology by regulating metabolic pathways and as a tumour suppressor (for review see [104]). It has been shown that HCV sequesters miR-122 from its endogenous mRNA targets, thereby leading to their derepression and liver carcinogenesis [105]. Several other miRNAs that have a dual role in both the HCV replication cycle and in liver disease development have been reported, underscoring the tight interplay between HCV and miRNAs in the liver (Figure 1).

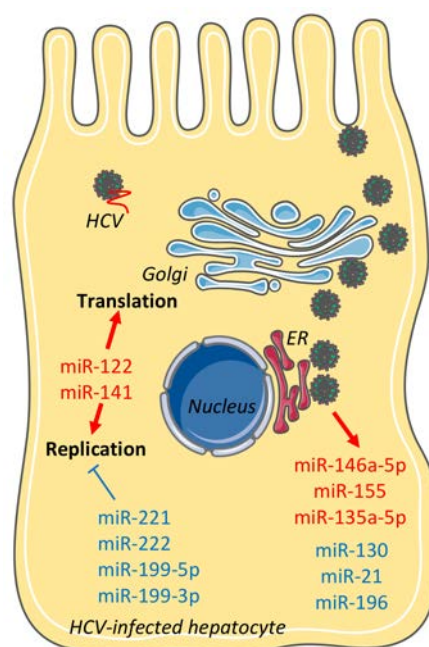


Figure 1. Impact of cellular miRNAs on the HCV life cycle and their contribution to HCV-induced HCC. Impact of individual miRNAs whose expression is modulated upon HCV infection on steps of the HCV life cycle (entry, translation, replication, assembly, and LVP release) are shown. miRNAs in blue display a proviral effect; miRNAs in red have an antiviral effect. HCV: hepatitis C virus. LVP: lipoviral particle. ER: endoplasmic reticulum. Golgi: Golgi apparatus. Images were adapted from SMART (Servier Medical Art).

A recent study that comprehensively analysed miRNAs modulated upon HCV infection showed that several of these miRNAs were known to regulate inflammation, fibrosis, and cancer development [106]. Among the miRNAs whose expression increased during HCV infection was miR-146a-5p, which has been associated with a modulation of the proteasome pathway, fatty acid, and anaerobic energetic metabolism. These regulatory pathways are both in favour of HCV infection (Figure 1) and liver disease development by promoting liver inflammation and HCC [106]. Furthermore, it has been shown that HCV infection modulates miR-196, miR-130, and miR-21 that are able to regulate type I IFN signalling pathways to overcome their antiviral activity [45] (Figure 1). These data are in line with miRNA expression profiles (differential expression of 19 miRNAs, including

miR-124b, miR-34c, and miR-23a) associated with HCV infection [41]. Analysis of targeted genes using infection-associated miRNAs revealed that the pathways related to the immune response, antigen presentation, the cell cycle, proteasome, and lipid metabolism, are activated in the HCV-infected liver, suggesting their implication in HCV-induced liver disease pathogenesis. Interestingly, some of these miRNAs also contribute to the modulation of HCV infection. For example, miR-141 that is upregulated in HCV-infected cells has been shown to downregulate the DLC-1 (Rho GTPase) tumour suppressor and to be required for HCV replication [44] (Figure 1). Finally, miR-199-5p/miR-199-3p, miR-221, and miR-222, whose expression is correlated with fibrosis in HCV-infected patients, have been shown to reduce HCV RNA replication by binding the stem loop II region of HCV 5' UTR [107]. Of note, while DAA therapy has been reported to modulate hepatic miRNA expression, no significant changes in the expression of miRNAs that have been previously associated with a pro- or antiviral effect on HCV were shown, except an increase in miR-122 expression [108].

3. Conclusions and Perspectives

3.1. ncRNAs as Novel Biomarkers for Detection of HCV-Induced HCC

By contributing to the regulation of the HCV life cycle, liver disease development, and carcinogenesis, ncRNAs play a major role in CHC. Most CHC patients are asymptomatic for many years, and HCC usually develops after several decades of HCV infection [5]. The long latency period between initial HCV infection and HCC development provides an important time window of opportunity for individuals to be monitored for disease progression and intervention. Since early diagnosis increases the chance of effective therapy, patients with cirrhosis are enrolled in periodic ultrasound-based surveillance programmes [109]. However, given the challenge of detecting small liver tumours using ultrasound and the absence of a robust HCC serum marker, reliable non-invasive biomarker(s) would be most helpful for determining HCC risk and/or detecting HCC at early stages.

Molecular signatures using ncRNA expression profiles have been described as potential predictive or prognostic biomarkers of HCC and circulating ncRNAs hold promise as biomarkers for the (early) detection of HCC. In association with alpha-fetoprotein (AFP, the currently most widely used diagnostic HCC serum marker), UCA1 and WRAP53 have been suggested as diagnostic and prognostic markers of HCV-induced HCC [72] (Figure 2). Indeed, a high expression of serum UCA1 was significantly associated with a high tumour grade, large tumour size, positive vascular invasion, and advanced TNM stage in HCC patients [110]. Furthermore, two other lncRNAs might hold promise as potential biomarkers for HCV-related HCC: MALAT1 and HEIH (Figure 2). Indeed, it has been reported that MALAT1 in combination with AFP serum levels might indicate a worse liver failure score in HCV-related HCC patients [111] and lncRNA HEIH expression in serum and exosomes appeared to be increased in HCV-related HCC patients in contrast to patients with CHC or HCV-induced cirrhosis [74]. Further studies using different patient cohorts are needed to assess the potential of lncRNAs as HCC biomarkers. Several panels of miRNAs have also been suggested as potential HCC biomarkers in HCV-infected patients. For example, Zekri et al. identified an miRNA panel composed of miR-122, miR-885-5p, and miR-29b in association with AFP as a new biomarker for the early detection of HCC in a normal population, while another miRNA panel composed of miR-122, miR-885-5p, miR-221, and miR-22 in association with AFP provided a high diagnostic accuracy for the early detection of HCC in cirrhotic patients [112]. Furthermore, the potential of circulating miR-15b and miR-122, as well as miR-182 and miR-150, have been suggested as biomarkers to assess HCC risk in cirrhotic HCV-infected patients [113] and for the detection of cirrhosis progression and HCC in a cohort of HCV-infected Egyptian patients [114], respectively. Moreover, Okajima et al. [115] showed that circulating miR-224 could be a novel biomarker for the detection of primary and recurrent HCC. Finally, a panel of nine liver-associated miRNAs (miR-21, miR-30c, miR-93, miR-122, miR-125b, miR-126, miR-130a, miR-193b, and miR-222) could discriminate healthy individuals from patients with HCV-related HCC [116]

(Figure 2). Further patient cohort studies are required to assess the utility of these miRNAs for CHC patient surveillance programmes.

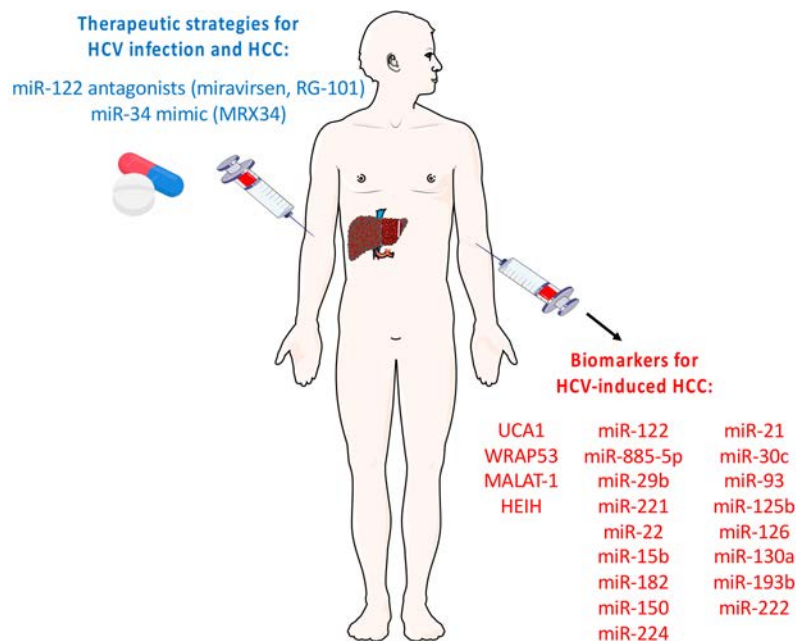


Figure 2. Schematic representation of the potential use of ncRNAs as therapeutic targets for HCV infection and HCC or biomarkers for HCV-induced HCC. MiR-122 antagonists miravirsen and RG-101 have been shown to lead to a dose-dependent reduction of viral RNA in CHC patients [108,109]. miR-34 mimic (MRX34, Mirna Therapeutics) has been administered to patients with primary liver cancer, but this trial was abrogated due to immune-related adverse effects. LncRNAs and miRNAs that have been suggested as biomarkers for HCV-induced HCC are also indicated [42,72,74,111]. HCV: hepatitis C virus. HCC: hepatocellular carcinoma. Images were adapted from SMART (Servier Medical Art).

3.2. ncRNAs as Novel Therapeutic Targets of HCV-Induced HCC

In addition to their potential role as biomarkers, ncRNAs may also represent therapeutic targets (Figure 2). Several studies demonstrated *in vivo* proof-of-concept of using ncRNA/ncRNA antagonists to modulate gene expression, including in the liver [117–119]. LncRNAs that act as transcriptional enhancers might be used to increase gene expression, as demonstrated by the use of “antagoNATs” (oligonucleotides or siRNA designed to inhibit NATs) [119]. Another therapeutic option is to target miRNAs by using miRNA antagonists/antisense oligonucleotides to sequester miRNAs. The feasibility of such strategies for targeting the human liver is demonstrated by clinical trials in CHC patients having shown a dose-dependent prolonged reduction of viral RNA in patients treated with miR-122 antagonists miravirsen or RG-101 [117,118] (Figure 2). Furthermore, another clinical trial tested a nanoliposome-incorporated miR-34 mimic (MRX34, Mirna Therapeutics) in patients with primary liver cancer (Figure 2). However, this trial was abrogated due to immune-related adverse effects. Further investigations are required to fully characterize the biological functions of ncRNAs and to understand the impact of ncRNA modulation in liver carcinogenesis in order to develop new clinical therapies.

Funding: The authors’ work is supported by Inserm, the University of Lyon, the University of Strasbourg, the French National Agency for Research on AIDS and Viral Hepatitis (ANRS), the French National Research Agency (EpiVirHep, RHU cirB-RNA), the Impulsion Program of the IDEXLYON, and the European Union (EU H2020-667273-HEPCAR). This work has been published under the framework of the LABEX ANR-10-LABX-0028_HEPSYS and benefits from funding from the state managed by the French National Research Agency as part of the Investments for the future program. K.H. is supported by a PhD fellowship from the IdEx program of the University of Strasbourg.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. The Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis c virus infection in 2015: A modelling study. *Lancet Gastroenterol. Hepatol.* **2017**, *2*, 161–176. [[CrossRef](#)]
2. Maucort-Boulch, D.; de Martel, C.; Franceschi, S.; Plummer, M. Fraction and incidence of liver cancer attributable to hepatitis b and c viruses worldwide. *Int. J. Cancer* **2018**, *142*, 2471–2477. [[CrossRef](#)] [[PubMed](#)]
3. Jeong, S.W.; Jang, J.Y.; Chung, R.T. Hepatitis c virus and hepatocarcinogenesis. *Clin. Mol. Hepatol.* **2012**, *18*, 347–356. [[CrossRef](#)] [[PubMed](#)]
4. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* **2015**, *136*, E359–E386. [[CrossRef](#)] [[PubMed](#)]
5. European Association for the Study of the Liver. Easl clinical practice guidelines: Management of hepatocellular carcinoma. *J. Hepatol.* **2018**, *69*, 182–236. [[CrossRef](#)] [[PubMed](#)]
6. Ganten, T.M.; Stauber, R.E.; Schott, E.; Malfertheiner, P.; Buder, R.; Galle, P.R.; Gohler, T.; Walther, M.; Koschny, R.; Gerken, G. Sorafenib in patients with hepatocellular carcinoma—results of the observational insight study. *Clin. Cancer Res.* **2017**, *23*, 5720–5728. [[CrossRef](#)] [[PubMed](#)]
7. Finn, R.S.; Merle, P.; Granito, A.; Huang, Y.H.; Bodoky, G.; Pracht, M.; Yokosuka, O.; Rosmorduc, O.; Gerolami, R.; Caparello, C.; et al. Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III resorce trial. *J. Hepatol.* **2018**, *69*, 353–358. [[CrossRef](#)] [[PubMed](#)]
8. Abou-Alfa, G.K.; Meyer, T.; Cheng, A.L.; El-Khoueiry, A.B.; Rimassa, L.; Ryoo, B.Y.; Cicin, I.; Merle, P.; Chen, Y.; Park, J.W.; et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N. Engl. J. Med.* **2018**, *379*, 54–63. [[CrossRef](#)] [[PubMed](#)]
9. Choo, Q.L.; Kuo, G.; Weiner, A.J.; Overby, L.R.; Bradley, D.W.; Houghton, M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **1989**, *244*, 359–362. [[CrossRef](#)] [[PubMed](#)]
10. Lindenbach, B.D.; Rice, C.M. Molecular biology of flaviviruses. *Adv. Virus Res.* **2003**, *59*, 23–61. [[PubMed](#)]
11. Chisari, F.V. Unscrambling hepatitis c virus-host interactions. *Nature* **2005**, *436*, 930–932. [[CrossRef](#)] [[PubMed](#)]
12. Tellinghuisen, T.L.; Rice, C.M. Interaction between hepatitis c virus proteins and host cell factors. *Curr. Opin. Microbiol.* **2002**, *5*, 419–427. [[CrossRef](#)]
13. El-Serag, H.B. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* **2012**, *142*, 1264–1273. [[CrossRef](#)] [[PubMed](#)]
14. Arzumanyan, A.; Reis, H.M.; Feitelson, M.A. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat. Rev. Cancer* **2013**, *13*, 123–135. [[CrossRef](#)] [[PubMed](#)]
15. Bandiera, S.; Billie Bian, C.; Hoshida, Y.; Baumert, T.F.; Zeisel, M.B. Chronic hepatitis c virus infection and pathogenesis of hepatocellular carcinoma. *Curr. Opin. Virol.* **2016**, *20*, 99–105. [[CrossRef](#)] [[PubMed](#)]
16. Shen, S.; Li, C.; Dai, M.; Yan, X. Induction of huh7 cell apoptosis by HCV core proteins via ck1 α pathway signaling pathway. *Mol. Med. Rep.* **2018**, *17*, 7559–7566. [[CrossRef](#)] [[PubMed](#)]
17. Kwak, J.; Choi, J.H.; Jang, K.L. Hepatitis c virus core overcomes all-trans retinoic acid-induced apoptosis in human hepatoma cells by inhibiting p14 expression via DNA methylation. *Oncotarget* **2017**, *8*, 85584–85598. [[CrossRef](#)] [[PubMed](#)]
18. Ray, R.B.; Steele, R.; Meyer, K.; Ray, R. Transcriptional repression of p53 promoter by hepatitis c virus core protein. *J. Biol. Chem.* **1997**, *272*, 10983–10986. [[CrossRef](#)] [[PubMed](#)]
19. Cho, J.; Baek, W.; Yang, S.; Chang, J.; Sung, Y.C.; Suh, M. Hcv core protein modulates rb pathway through prb down-regulation and e2f-1 up-regulation. *Biochim. Biophys. Acta* **2001**, *1538*, 59–66. [[CrossRef](#)]
20. Noell, B.C.; Besur, S.V.; deLemos, A.S. Changing the face of hepatitis c management—The design and development of sofosbuvir. *Drug Des. Dev. Ther.* **2015**, *9*, 2367–2374. [[CrossRef](#)]
21. Nahon, P.; Layese, R.; Bourcier, V.; Cagnot, C.; Marcellin, P.; Guyader, D.; Pol, S.; Larrey, D.; De Ledinghen, V.; Ouzan, D.; et al. Incidence of hepatocellular carcinoma after direct antiviral therapy for HCV in patients with cirrhosis included in surveillance programs. *Gastroenterology* **2018**. [[CrossRef](#)] [[PubMed](#)]
22. Aleman, S.; Rahbin, N.; Weiland, O.; Davidsdottir, L.; Hedenstierna, M.; Rose, N.; Verbaan, H.; Stal, P.; Carlsson, T.; Norrgren, H.; et al. A risk for hepatocellular carcinoma persists long-term after sustained

- virologic response in patients with hepatitis c-associated liver cirrhosis. *Clin. Infect. Dis.* **2013**, *57*, 230–236. [[CrossRef](#)] [[PubMed](#)]
23. Reig, M.; Marino, Z.; Perello, C.; Inarrairaegui, M.; Ribeiro, A.; Lens, S.; Diaz, A.; Vilana, R.; Darnell, A.; Varela, M.; et al. Unexpected high rate of early tumor recurrence in patients with HCV-related hcc undergoing interferon-free therapy. *J. Hepatol.* **2016**, *65*, 719–726. [[CrossRef](#)] [[PubMed](#)]
 24. Conti, F.; Buonfiglioli, F.; Scuteri, A.; Crespi, C.; Bolondi, L.; Caraceni, P.; Foschi, F.G.; Lenzi, M.; Mazzella, G.; Verucchi, G.; et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J. Hepatol.* **2016**, *65*, 727–733. [[CrossRef](#)] [[PubMed](#)]
 25. Kozbial, K.; Moser, S.; Schwarzer, R.; Laferl, H.; Al-Zoairy, R.; Stauber, R.; Stattemayer, A.F.; Beinhardt, S.; Graziadei, I.; Freissmuth, C.; et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J. Hepatol.* **2016**, *65*, 856–858. [[CrossRef](#)] [[PubMed](#)]
 26. Hayes, C.N.; Zhang, P.; Zhang, Y.; Chayama, K. Molecular mechanisms of hepatocarcinogenesis following sustained virological response in patients with chronic hepatitis c virus infection. *Viruses* **2018**, *10*, 531. [[CrossRef](#)] [[PubMed](#)]
 27. Pol, S. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: Data from three anrs cohorts. *J. Hepatol.* **2016**, *65*, 734–740. [[CrossRef](#)]
 28. Nishibatake Kinoshita, M.; Minami, T.; Tateishi, R.; Wake, T.; Nakagomi, R.; Fujiwara, N.; Sato, M.; Uchino, K.; Enooku, K.; Nakagawa, H.; et al. Impact of direct-acting antivirals on early recurrence of HCV-related hcc: Comparison with interferon-based therapy. *J. Hepatol.* **2018**. [[CrossRef](#)] [[PubMed](#)]
 29. Nagata, H.; Nakagawa, M.; Asahina, Y.; Sato, A.; Asano, Y.; Tsunoda, T.; Miyoshi, M.; Kaneko, S.; Otani, S.; Kawai-Kitahata, F.; et al. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis c. *J. Hepatol.* **2017**, *67*, 933–939. [[CrossRef](#)] [[PubMed](#)]
 30. Kanwal, F.; Kramer, J.; Asch, S.M.; Chayanupatkul, M.; Cao, Y.; El-Serag, H.B. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* **2017**, *153*, 996–1005. [[CrossRef](#)] [[PubMed](#)]
 31. Cabibbo, G.; Petta, S.; Calvaruso, V.; Cacciola, I.; Cannavo, M.R.; Madonia, S.; Distefano, M.; Larocca, L.; Prestileo, T.; Tine, F.; et al. Is early recurrence of hepatocellular carcinoma in HCV cirrhotic patients affected by treatment with direct-acting antivirals? A prospective multicentre study. *Aliment. Pharmacol. Ther.* **2017**, *46*, 688–695. [[CrossRef](#)] [[PubMed](#)]
 32. Calvaruso, V.; Cabibbo, G.; Cacciola, I.; Petta, S.; Madonia, S.; Bellia, A.; Tine, F.; Distefano, M.; Licata, A.; Giannitrapani, L.; et al. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. *Gastroenterology* **2018**, *155*, 411–421. [[CrossRef](#)] [[PubMed](#)]
 33. Virlogeux, V.; Pradat, P.; Hartig-Lavie, K.; Bailly, F.; Maynard, M.; Ouziel, G.; Poinot, D.; Lebosse, F.; Ecochard, M.; Radenne, S.; et al. Direct-acting antiviral therapy decreases hepatocellular carcinoma recurrence rate in cirrhotic patients with chronic hepatitis c. *Liver Int.* **2017**, *37*, 1122–1127. [[CrossRef](#)] [[PubMed](#)]
 34. Levrero, M.; Zucman-Rossi, J. Mechanisms of HBV-induced hepatocellular carcinoma. *J. Hepatol.* **2016**, *64*, S84–S101. [[CrossRef](#)] [[PubMed](#)]
 35. Hayes, C.N.; Chayama, K. MicroRNAs as biomarkers for liver disease and hepatocellular carcinoma. *Int. J. Mol. Sci.* **2016**, *17*, 280. [[CrossRef](#)] [[PubMed](#)]
 36. Salviano-Silva, A.; Lobo-Alves, S.C.; Almeida, R.C.; Malheiros, D.; Petzl-Erler, M.L. Besides pathology: Long non-coding RNA in cell and tissue homeostasis. *Noncoding RNA* **2018**, *4*, 3. [[CrossRef](#)] [[PubMed](#)]
 37. Lucafo, M.; De Iudicibus, S.; Di Silvestre, A.; Pelin, M.; Candussio, L.; Martellosi, S.; Tommasini, A.; Piscianz, E.; Ventura, A.; Decorti, G. Long noncoding RNA gas5: A novel marker involved in glucocorticoid response. *Curr. Mol. Med.* **2015**, *15*, 94–99. [[CrossRef](#)] [[PubMed](#)]
 38. Esteller, M. Non-coding RNAs in human disease. *Nat. Rev. Genet.* **2011**, *12*, 861–874. [[CrossRef](#)] [[PubMed](#)]
 39. Wong, C.M.; Tsang, F.H.; Ng, I.O. Non-coding RNAs in hepatocellular carcinoma: Molecular functions and pathological implications. *Nat. Rev. Gastroenterol. Hepatol.* **2018**, *15*, 137–151. [[CrossRef](#)] [[PubMed](#)]
 40. Kapranov, P.; Cheng, J.; Dike, S.; Nix, D.A.; Dutttagupta, R.; Willingham, A.T.; Stadler, P.F.; Hertel, J.; Hackermuller, J.; Hofacker, I.L.; et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science* **2007**, *316*, 1484–1488. [[CrossRef](#)] [[PubMed](#)]

41. Ura, S.; Honda, M.; Yamashita, T.; Ueda, T.; Takatori, H.; Nishino, R.; Sunakozaka, H.; Sakai, Y.; Horimoto, K.; Kaneko, S. Differential microRNA expression between hepatitis b and hepatitis c leading disease progression to hepatocellular carcinoma. *Hepatology* **2009**, *49*, 1098–1112. [[CrossRef](#)] [[PubMed](#)]
42. Diaz, G.; Melis, M.; Tice, A.; Kleiner, D.E.; Mishra, L.; Zamboni, F.; Farci, P. Identification of microRNAs specifically expressed in hepatitis c virus-associated hepatocellular carcinoma. *Int. J. Cancer* **2013**, *133*, 816–824. [[CrossRef](#)] [[PubMed](#)]
43. Hou, W.; Bonkovsky, H.L. Non-coding RNAs in hepatitis c-induced hepatocellular carcinoma: Dysregulation and implications for early detection, diagnosis and therapy. *World J. Gastroenterol.* **2013**, *19*, 7836–7845. [[CrossRef](#)] [[PubMed](#)]
44. Kumar, A. MicroRNA in HCV infection and liver cancer. *Biochim. Biophys. Acta* **2011**, *1809*, 694–699. [[CrossRef](#)] [[PubMed](#)]
45. Lee, C.H.; Kim, J.H.; Lee, S.W. The role of microRNAs in hepatitis c virus replication and related liver diseases. *J. Microbiol.* **2014**, *52*, 445–451. [[CrossRef](#)] [[PubMed](#)]
46. Iyer, M.K.; Niknafs, Y.S.; Malik, R.; Singhal, U.; Sahu, A.; Hosono, Y.; Barrette, T.R.; Prensner, J.R.; Evans, J.R.; Zhao, S.; et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat. Genet.* **2015**, *47*, 199–208. [[CrossRef](#)] [[PubMed](#)]
47. Shi, L.; Peng, F.; Tao, Y.; Fan, X.; Li, N. Roles of long noncoding RNAs in hepatocellular carcinoma. *Virus Res.* **2016**, *223*, 131–139. [[CrossRef](#)] [[PubMed](#)]
48. Carninci, P.; Kasukawa, T.; Katayama, S.; Gough, J.; Frith, M.C.; Maeda, N.; Oyama, R.; Ravasi, T.; Lenhard, B.; Wells, C.; et al. The transcriptional landscape of the mammalian genome. *Science* **2005**, *309*, 1559–1563. [[CrossRef](#)] [[PubMed](#)]
49. Derrien, T.; Johnson, R.; Bussotti, G.; Tanzer, A.; Djebali, S.; Tilgner, H.; Guernec, G.; Martin, D.; Merkel, A.; Knowles, D.G.; et al. The gencode v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. *Genome Res.* **2012**, *22*, 1775–1789. [[CrossRef](#)] [[PubMed](#)]
50. Ma, L.; Bajic, V.B.; Zhang, Z. On the classification of long non-coding RNAs. *RNA Biol.* **2013**, *10*, 925–933. [[CrossRef](#)] [[PubMed](#)]
51. Han, L.; Ma, P.; Liu, S.M.; Zhou, X. Circulating long noncoding RNA gas5 as a potential biomarker in breast cancer for assessing the surgical effects. *Tumour Biol.* **2016**, *37*, 6847–6854. [[CrossRef](#)] [[PubMed](#)]
52. Han, Y.; Zhou, L.; Wu, T.; Huang, Y.; Cheng, Z.; Li, X.; Sun, T.; Zhou, Y.; Du, Z. Downregulation of lncRNA-MALAT1 affects proliferation and the expression of stemness markers in glioma stem cell line SHG139S. *Cell. Mol. Neurobiol.* **2016**, *36*, 1097–1107. [[CrossRef](#)] [[PubMed](#)]
53. He, Y.; Meng, X.M.; Huang, C.; Wu, B.M.; Zhang, L.; Lv, X.W.; Li, J. Long noncoding RNAs: Novel insights into hepatocellular carcinoma. *Cancer Lett.* **2014**, *344*, 20–27. [[CrossRef](#)] [[PubMed](#)]
54. Klingenberg, M.; Matsuda, A.; Diederichs, S.; Patel, T. Non-coding RNA in hepatocellular carcinoma: Mechanisms, biomarkers and therapeutic targets. *J. Hepatol.* **2017**, *67*, 603–618. [[CrossRef](#)] [[PubMed](#)]
55. Ponting, C.P.; Oliver, P.L.; Reik, W. Evolution and functions of long noncoding RNAs. *Cell* **2009**, *136*, 629–641. [[CrossRef](#)] [[PubMed](#)]
56. Salzman, J. Circular RNA expression: Its potential regulation and function. *Trends Genet.* **2016**, *32*, 309–316. [[CrossRef](#)] [[PubMed](#)]
57. Poliseno, L.; Salmena, L.; Zhang, J.; Carver, B.; Haveman, W.J.; Pandolfi, P.P. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature* **2010**, *465*, 1033–1038. [[CrossRef](#)] [[PubMed](#)]
58. Chan, J.J.; Tay, Y. Noncoding RNA: RNA regulatory networks in cancer. *Int. J. Mol. Sci.* **2018**, *19*, 1310. [[CrossRef](#)] [[PubMed](#)]
59. Huang, J.L.; Zheng, L.; Hu, Y.W.; Wang, Q. Characteristics of long non-coding RNA and its relation to hepatocellular carcinoma. *Carcinogenesis* **2014**, *35*, 507–514. [[CrossRef](#)] [[PubMed](#)]
60. Yang, X.; Xie, X.; Xiao, Y.F.; Xie, R.; Hu, C.J.; Tang, B.; Li, B.S.; Yang, S.M. The emergence of long non-coding RNAs in the tumorigenesis of hepatocellular carcinoma. *Cancer Lett.* **2015**, *360*, 119–124. [[CrossRef](#)] [[PubMed](#)]
61. El Khodiry, A.; Afify, M.; El Tayebi, H.M. Behind the curtain of non-coding RNAs; long non-coding RNAs regulating hepatocarcinogenesis. *World J. Gastroenterol.* **2018**, *24*, 549–572. [[CrossRef](#)] [[PubMed](#)]
62. Mehra, M.; Chauhan, R. Long noncoding RNAs as a key player in hepatocellular carcinoma. *Biomark. Cancer* **2017**, *9*. [[CrossRef](#)] [[PubMed](#)]

63. Lanzafame, M.; Bianco, G.; Terracciano, L.M.; Ng, C.K.Y.; Piscuoglio, S. The role of long non-coding RNAs in hepatocarcinogenesis. *Int. J. Mol. Sci.* **2018**, *19*, 682. [[CrossRef](#)] [[PubMed](#)]
64. Cui, H.; Zhang, Y.; Zhang, Q.; Chen, W.; Zhao, H.; Liang, J. A comprehensive genome-wide analysis of long noncoding RNA expression profile in hepatocellular carcinoma. *Cancer Med.* **2017**, *6*, 2932–2941. [[CrossRef](#)] [[PubMed](#)]
65. Ishibashi, M.; Kogo, R.; Shibata, K.; Sawada, G.; Takahashi, Y.; Kurashige, J.; Akiyoshi, S.; Sasaki, S.; Iwaya, T.; Sudo, T.; et al. Clinical significance of the expression of long non-coding RNA hotair in primary hepatocellular carcinoma. *Oncol. Rep.* **2013**, *29*, 946–950. [[CrossRef](#)] [[PubMed](#)]
66. Yang, Z.; Zhou, L.; Wu, L.M.; Lai, M.C.; Xie, H.Y.; Zhang, F.; Zheng, S.S. Overexpression of long non-coding RNA hotair predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. *Ann. Surg. Oncol.* **2011**, *18*, 1243–1250. [[CrossRef](#)] [[PubMed](#)]
67. Geng, Y.J.; Xie, S.L.; Li, Q.; Ma, J.; Wang, G.Y. Large intervening non-coding RNA hotair is associated with hepatocellular carcinoma progression. *J. Int. Med. Res.* **2011**, *39*, 2119–2128. [[CrossRef](#)] [[PubMed](#)]
68. Wang, B.G.; Lv, Z.; Ding, H.X.; Fang, X.X.; Wen, J.; Xu, Q.; Yuan, Y. The association of lncRNA-hulc polymorphisms with hepatocellular cancer risk and prognosis. *Gene* **2018**, *670*, 148–154. [[CrossRef](#)] [[PubMed](#)]
69. Guo, J.; Hao, C.; Wang, C.; Li, L. Long noncoding RNA PVT1 modulates hepatocellular carcinoma cell proliferation and apoptosis by recruiting EZH2. *Cancer Cell Int.* **2018**, *18*, 98. [[CrossRef](#)] [[PubMed](#)]
70. Zhang, H.; Zhu, C.; Zhao, Y.; Li, M.; Wu, L.; Yang, X.; Wan, X.; Wang, A.; Zhang, M.Q.; Sang, X.; et al. Long non-coding RNA expression profiles of hepatitis c virus-related dysplasia and hepatocellular carcinoma. *Oncotarget* **2015**, *6*, 43770–43778. [[CrossRef](#)] [[PubMed](#)]
71. Zhang, Q.; Matsuura, K.; Kleiner, D.E.; Zamboni, F.; Alter, H.J.; Farci, P. Analysis of long noncoding RNA expression in hepatocellular carcinoma of different viral etiology. *J. Transl. Med.* **2016**, *14*, 328. [[CrossRef](#)] [[PubMed](#)]
72. Kamel, M.M.; Matboli, M.; Sallam, M.; Montasser, I.F.; Saad, A.S.; El-Tawdi, A.H.F. Investigation of long noncoding RNAs expression profile as potential serum biomarkers in patients with hepatocellular carcinoma. *Transl. Res.* **2016**, *168*, 134–145. [[CrossRef](#)] [[PubMed](#)]
73. Xiao, J.N.; Yan, T.H.; Yu, R.M.; Gao, Y.; Zeng, W.L.; Lu, S.W.; Que, H.X.; Liu, Z.P.; Jiang, J.H. Long non-coding RNA UCA1 regulates the expression of Snail2 by Mir-203 to promote hepatocellular carcinoma progression. *J. Cancer Res. Clin. Oncol.* **2017**, *143*, 981–990. [[CrossRef](#)] [[PubMed](#)]
74. Zhang, C.; Yang, X.; Qi, Q.; Gao, Y.; Wei, Q.; Han, S. Lncrna-heih in serum and exosomes as a potential biomarker in the HCV-related hepatocellular carcinoma. *Cancer Biomark.* **2018**, *21*, 651–659. [[CrossRef](#)] [[PubMed](#)]
75. Stepanov, G.A.; Filippova, J.A.; Komissarov, A.B.; Kuligina, E.V.; Richter, V.A.; Semenov, D.V. Regulatory role of small nucleolar RNAs in human diseases. *Biomed. Res. Int.* **2015**, *2015*, 206849. [[CrossRef](#)] [[PubMed](#)]
76. Kishore, S.; Stamm, S. The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2c. *Science* **2006**, *311*, 230–232. [[CrossRef](#)] [[PubMed](#)]
77. Ender, C.; Krek, A.; Friedlander, M.R.; Beitzinger, M.; Weinmann, L.; Chen, W.; Pfeffer, S.; Rajewsky, N.; Meister, G. A human snoRNA with microRNA-like functions. *Mol. Cell* **2008**, *32*, 519–528. [[CrossRef](#)] [[PubMed](#)]
78. Cavaille, J.; Nicoloso, M.; Bachellerie, J.P. Targeted ribose methylation of RNA in vivo directed by tailored antisense RNA guides. *Nature* **1996**, *383*, 732–735. [[CrossRef](#)] [[PubMed](#)]
79. Ganot, P.; Bortolin, M.L.; Kiss, T. Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. *Cell* **1997**, *89*, 799–809. [[CrossRef](#)]
80. Scott, M.S.; Ono, M. From snoRNA to miRNA: Dual function regulatory non-coding RNAs. *Biochimie* **2011**, *93*, 1987–1992. [[CrossRef](#)] [[PubMed](#)]
81. King, T.H.; Liu, B.; McCully, R.R.; Fournier, M.J. Ribosome structure and activity are altered in cells lacking snorncps that form pseudouridines in the peptidyl transferase center. *Mol. Cell* **2003**, *11*, 425–435. [[CrossRef](#)]
82. Dieci, G.; Preti, M.; Montanini, B. Eukaryotic snoRNAs: A paradigm for gene expression flexibility. *Genomics* **2009**, *94*, 83–88. [[CrossRef](#)] [[PubMed](#)]
83. Baral, D.; Wu, L.; Katwal, G.; Yan, X.; Wang, Y.; Ye, Q. Clinical significance and biological roles of small nucleolar RNAs in hepatocellular carcinoma. *Biomed. Rep.* **2018**, *8*, 319–324. [[CrossRef](#)] [[PubMed](#)]
84. Xu, G.; Yang, F.; Ding, C.L.; Zhao, L.J.; Ren, H.; Zhao, P.; Wang, W.; Qi, Z.T. Small nucleolar RNA 113-1 suppresses tumorigenesis in hepatocellular carcinoma. *Mol. Cancer* **2014**, *13*, 216. [[CrossRef](#)] [[PubMed](#)]

85. Fang, X.; Yang, D.; Luo, H.; Wu, S.; Dong, W.; Xiao, J.; Yuan, S.; Ni, A.; Zhang, K.J.; Liu, X.Y.; et al. SNORD126 promotes HCC and CRC cell growth by activating the PI3K-AKT pathway through FGFR2. *J. Mol. Cell Biol.* **2017**, *9*, 243–255. [[CrossRef](#)] [[PubMed](#)]
86. Siomi, M.C.; Sato, K.; Pezic, D.; Aravin, A.A. Piwi-interacting small RNAs: The vanguard of genome defence. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 246–258. [[CrossRef](#)] [[PubMed](#)]
87. Ng, K.W.; Anderson, C.; Marshall, E.A.; Minatel, B.C.; Enfield, K.S.; Saprunoff, H.L.; Lam, W.L.; Martinez, V.D. Piwi-interacting RNAs in cancer: Emerging functions and clinical utility. *Mol. Cancer* **2016**, *15*, 5. [[CrossRef](#)] [[PubMed](#)]
88. Rizzo, F.; Rinaldi, A.; Marchese, G.; Coviello, E.; Sellitto, A.; Cordella, A.; Giurato, G.; Nassa, G.; Ravo, M.; Tarallo, R.; et al. Specific patterns of piwi-interacting small noncoding RNA expression in dysplastic liver nodules and hepatocellular carcinoma. *Oncotarget* **2016**, *7*, 54650–54661. [[CrossRef](#)] [[PubMed](#)]
89. Law, P.T.; Qin, H.; Ching, A.K.; Lai, K.P.; Co, N.N.; He, M.; Lung, R.W.; Chan, A.W.; Chan, T.F.; Wong, N. Deep sequencing of small RNA transcriptome reveals novel non-coding RNAs in hepatocellular carcinoma. *J. Hepatol.* **2013**, *58*, 1165–1173. [[CrossRef](#)] [[PubMed](#)]
90. Bartel, D.P. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* **2004**, *116*, 281–297. [[CrossRef](#)]
91. He, L.; Hannon, G.J. MicroRNAs: Small RNAs with a big role in gene regulation. *Nat. Rev. Genet.* **2004**, *5*, 522–531. [[CrossRef](#)] [[PubMed](#)]
92. Krol, J.; Loedige, I.; Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* **2010**, *11*, 597–610. [[CrossRef](#)] [[PubMed](#)]
93. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 509–524. [[CrossRef](#)] [[PubMed](#)]
94. Heyns, M.; Kovalchuk, O. Non-coding RNAs including miRNAs, piRNAs, and tRNAs in human cancer. *Oncotarget* **2015**, *6*, 23055–23057. [[CrossRef](#)] [[PubMed](#)]
95. Hwang, H.W.; Mendell, J.T. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br. J. Cancer* **2006**, *94*, 776–780. [[CrossRef](#)] [[PubMed](#)]
96. Mendell, J.T. MicroRNAs: Critical regulators of development, cellular physiology and malignancy. *Cell Cycle* **2005**, *4*, 1179–1184. [[CrossRef](#)] [[PubMed](#)]
97. Romano, G.; Veneziano, D.; Acunzo, M.; Croce, C.M. Small non-coding RNA and cancer. *Carcinogenesis* **2017**, *38*, 485–491. [[CrossRef](#)] [[PubMed](#)]
98. Murakami, Y.; Yasuda, T.; Saigo, K.; Urashima, T.; Toyoda, H.; Okanou, T.; Shimotohno, K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* **2006**, *25*, 2537–2545. [[CrossRef](#)] [[PubMed](#)]
99. Pezzuto, F.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. The role of circulating free DNA and microRNA in non-invasive diagnosis of HBV- and HCV-related hepatocellular carcinoma. *Int. J. Mol. Sci.* **2018**, *19*. [[CrossRef](#)]
100. Pineau, P.; Volinia, S.; McJunkin, K.; Marchio, A.; Battiston, C.; Terris, B.; Mazzaferro, V.; Lowe, S.W.; Croce, C.M.; Dejean, A. Mir-221 overexpression contributes to liver tumorigenesis. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 264–269. [[CrossRef](#)] [[PubMed](#)]
101. An, F.; Oлару, A.V.; Mezey, E.; Xie, Q.; Li, L.; Piontek, K.B.; Selaru, F.M. MicroRNA-224 induces g1/s checkpoint release in liver cancer. *J. Clin. Med.* **2015**, *4*, 1713–1728. [[CrossRef](#)] [[PubMed](#)]
102. Zhang, Y.; Wei, W.; Cheng, N.; Wang, K.; Li, B.; Jiang, X.; Sun, S. Hepatitis c virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating wnt signaling. *Hepatology* **2012**, *56*, 1631–1640. [[CrossRef](#)] [[PubMed](#)]
103. Van Renne, N.; Roca Suarez, A.A.; Duong, F.H.T.; Gondeau, C.; Calabrese, D.; Fontaine, N.; Ababsa, A.; Bandiera, S.; Croonenborghs, T.; Pochet, N.; et al. Mir-135a-5p-mediated downregulation of protein tyrosine phosphatase receptor delta is a candidate driver of HCV-associated hepatocarcinogenesis. *Gut* **2018**, *67*, 953–962. [[CrossRef](#)] [[PubMed](#)]
104. Bandiera, S.; Pfeffer, S.; Baumert, T.F.; Zeisel, M.B. Mir-122—a key factor and therapeutic target in liver disease. *J. Hepatol.* **2015**, *62*, 448–457. [[CrossRef](#)] [[PubMed](#)]
105. Luna, J.M.; Scheel, T.K.; Danino, T.; Shaw, K.S.; Mele, A.; Fak, J.J.; Nishiuchi, E.; Takacs, C.N.; Catanese, M.T.; de Jong, Y.P.; et al. Hepatitis c virus RNA functionally sequesters mir-122. *Cell* **2015**, *160*, 1099–1110. [[CrossRef](#)] [[PubMed](#)]

106. Bandiera, S.; Pernot, S.; El Saghire, H.; Durand, S.C.; Thumann, C.; Crouchet, E.; Ye, T.; Fofana, I.; Oudot, M.A.; Barths, J.; et al. Hepatitis c virus-induced upregulation of microRNA Mir-146a-5p in hepatocytes promotes viral infection and deregulates metabolic pathways associated with liver disease pathogenesis. *J. Virol.* **2016**, *90*, 6387–6400. [[CrossRef](#)] [[PubMed](#)]
107. Murakami, Y.; Aly, H.H.; Tajima, A.; Inoue, I.; Shimotohno, K. Regulation of the hepatitis c virus genome replication by Mir-199a. *J. Hepatol.* **2009**, *50*, 453–460. [[CrossRef](#)] [[PubMed](#)]
108. Meissner, E.G.; Kohli, A.; Virtaneva, K.; Sturdevant, D.; Martens, C.; Porcella, S.F.; McHutchison, J.G.; Masur, H.; Kottlilil, S. Achieving sustained virologic response after interferon-free hepatitis c virus treatment correlates with hepatic interferon gene expression changes independent of cirrhosis. *J. Viral Hepat.* **2016**, *23*, 496–505. [[CrossRef](#)] [[PubMed](#)]
109. Della Corte, C.; Triolo, M.; Iavarone, M.; Sangiovanni, A. Early diagnosis of liver cancer: An appraisal of international recommendations and future perspectives. *Liver Int.* **2016**, *36*, 166–176. [[CrossRef](#)] [[PubMed](#)]
110. Zheng, Z.K.; Pang, C.; Yang, Y.; Duan, Q.; Zhang, J.; Liu, W.C. Serum long noncoding RNA urothelial carcinoma-associated 1: A novel biomarker for diagnosis and prognosis of hepatocellular carcinoma. *J. Int. Med. Res.* **2018**, *46*, 348–356. [[CrossRef](#)] [[PubMed](#)]
111. Toraih, E.A.; Ellawindy, A.; Fala, S.Y.; Al Ageeli, E.; Gouda, N.S.; Fawzy, M.S.; Hosny, S. Oncogenic long noncoding RNA MALAT1 and HCV-related hepatocellular carcinoma. *Biomed. Pharmacother.* **2018**, *102*, 653–669. [[CrossRef](#)] [[PubMed](#)]
112. Zekri, A.N.; Youssef, A.S.; El-Desouky, E.D.; Ahmed, O.S.; Lotfy, M.M.; Nassar, A.A.; Bahnassey, A.A. Serum microRNA panels as potential biomarkers for early detection of hepatocellular carcinoma on top of HCV infection. *Tumour Biol.* **2016**, *37*, 12273–12286. [[CrossRef](#)] [[PubMed](#)]
113. Huang, Y.H.; Liang, K.H.; Chien, R.N.; Hu, T.H.; Lin, K.H.; Hsu, C.W.; Lin, C.L.; Pan, T.L.; Ke, P.Y.; Yeh, C.T. A circulating microRNA signature capable of assessing the risk of hepatocellular carcinoma in cirrhotic patients. *Sci. Rep.* **2017**, *7*, 523. [[CrossRef](#)] [[PubMed](#)]
114. Shaheen, N.M.H.; Zayed, N.; Riad, N.M.; Tamim, H.H.; Shahin, R.M.H.; Labib, D.A.; SM, E.L.; Moneim, R.A.; Yosry, A.; Khalifa, R.H. Role of circulating mir-182 and mir-150 as biomarkers for cirrhosis and hepatocellular carcinoma post HCV infection in egyptian patients. *Virus Res.* **2018**. [[CrossRef](#)] [[PubMed](#)]
115. Okajima, W.; Komatsu, S.; Ichikawa, D.; Miyamae, M.; Kawaguchi, T.; Hirajima, S.; Ohashi, T.; Imamura, T.; Kiuchi, J.; Arita, T.; et al. Circulating microRNA profiles in plasma: Identification of mir-224 as a novel diagnostic biomarker in hepatocellular carcinoma independent of hepatic function. *Oncotarget* **2016**, *7*, 53820–53836. [[CrossRef](#)] [[PubMed](#)]
116. Ali, H.E.A.; Abdel Hameed, R.; Effat, H.; Ahmed, E.K.; Atef, A.A.; Sharawi, S.K.; Ali, M.; Abd Elmageed, Z.Y.; Abdel Wahab, A.H. Circulating microRNAs panel as a diagnostic tool for discrimination of HCV-associated hepatocellular carcinoma. *Clin. Res. Hepatol. Gastroenterol.* **2017**, *41*, e51–e62. [[CrossRef](#)] [[PubMed](#)]
117. Janssen, H.L.; Reesink, H.W.; Lawitz, E.J.; Zeuzem, S.; Rodriguez-Torres, M.; Patel, K.; van der Meer, A.J.; Patick, A.K.; Chen, A.; Zhou, Y.; et al. Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* **2013**, *368*, 1685–1694. [[CrossRef](#)] [[PubMed](#)]
118. van der Ree, M.H.; de Vree, J.M.; Stelma, F.; Willemse, S.; van der Valk, M.; Rietdijk, S.; Molenkamp, R.; Schinkel, J.; van Nuenen, A.C.; Beuers, U.; et al. Safety, tolerability, and antiviral effect of rg-101 in patients with chronic hepatitis c: A phase 1b, double-blind, randomised controlled trial. *Lancet* **2017**, *389*, 709–717. [[CrossRef](#)]
119. Wahlestedt, C. Targeting long non-coding RNA to therapeutically upregulate gene expression. *Nat. Rev. Drug Discov.* **2013**, *12*, 433–446. [[CrossRef](#)] [[PubMed](#)]



IX. CURRICULUM VITAE

General information

Full Name: Katharina HERZOG
Date of birth: September 1st 1989
Place of birth: Lahr/Schwarzwald, Germany
Nationality: German
Address: Wilhelm-Bartelt-Str.7, 77749 Hohberg, Germany
Phone: +49 (0) 176/ 322 766 11

Personalia

Katharina Herzog was born in Lahr/Schwarzwald, Germany on September 1st, 1989. She completed her secondary education at the Scheffel-Gymnasium in Lahr/Schwarzwald with the “general qualification for university entrance”. She continued to study Biology, obtaining her degree of Bachelor and Master of Science (Biology) in 2014 and 2016, respectively. Captivated by the subject of virology, she spent a large part of her last year at the university to work as a scientific assistant at the university hospital in Freiburg i.Br., Germany in the group of Prof. Robert Thimme obtaining technical and practical insides.

In an effort to increase her knowledge and acquire international experience, she then moved to Strasbourg where she obtained a competitive IDEX scholarship of the University of Strasbourg for a PhD fellowship. At INSERM UMR1110, the Institute of Viral and Liver Disease headed by Prof. Dr. Thomas Baumert, she uncovered a host factor that is modulating hepatitis C virus (HCV) morphogenesis and infectivity. She further characterized changes in the circadian clock upon HCV infection and identified cellular proteins and host factors bound to the HCV genome.

Scientific output

Original publications

2019 **Herzog K***, Bandiera S*, Pernot S, Fauvelle C, Jühling F, Weiss A, Bull A, Durand SC, Chane-Woon-Ming B, Pfeiffer S, Mercey M, Lerat H, Meunier JC, Raffelsberger W, Brino L, Baumert TF, Zeisel MB (*contributed equally). Functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as a host factor modulating hepatitis C virus morphogenesis and infectivity. Original Article. *Gut* 2019. pii: gutjnl-2018-317423. doi: 10.1136/gutjnl-2018-317423. Epub ahead of print May 10. *Authors contributed equally. Impact factor 2018: 17,016.

Review article

2018 Plissonnier ML, **Herzog K**, Levrero M, Zeisel MB. Non-coding RNA and HCV-induced HCC. *Viruses* 2018, 10 (11): 591.

Oral communications

2019 **Herzog K**⁺, Bandiera S, Pernot S, Fauvelle C, Jühling F, Weiss A, Bull A, Durand SC, Chane-Woon-Ming B, Pfeffer S, Mercey M, Lerat H, Meunier JC, Raffelsberger W, Brino L, Baumert TF, Zeisel MB (+presenter). Functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as a host factor modulating hepatitis C virus morphogenesis and infectivity. *Réunion du réseau national hépatites de l'ANRS (AC 42 groupe « métabolisme lipidique »)*, April 3, 2019, Paris, France.

2018 Bandiera S, Pernot S, **Herzog K**⁺, Fauvelle C, Weiss A, Durand SC, Raffelsberger W, Brino L, Baumert TF, Zeisel MB (+presenter). A microRNA Screen uncovers O-Linked N-Acetylglucosamine Transferase as a Host Factor involved in Hepatitis C Virus Morphogenesis. *18ème réunion du réseau national hépatites de l'ANRS, March 12-13, 2018, Paris, France*.

2018 Bandiera S, Pernot S, **Herzog K**⁺, Fauvelle C, Weiss A, Durand SC, Raffelsberger W, Brino L, Baumert TF, Zeisel MB (+presenter). A microRNA Screen uncovers O-Linked N-Acetylglucosamine Transferase as a Host Factor involved in Hepatitis C Virus Morphogenesis. *Génomique fonctionnelle du Foie (GFF)*, March 14-16, 2018, Lyon, France.

2018 Bandiera S, Pernot S, **Herzog K**⁺, Fauvelle C, Weiss A, Durand SC, Raffelsberger W, Brino L, Baumert TF, Zeisel MB (+presenter). A microRNA Screen uncovers O-Linked N-Acetylglucosamine Transferase as a Host Factor involved in Hepatitis C Virus Morphogenesis. *International Liver Congress (ILC, EASL)*, April 11-15, 2018, Paris, France.

Key skills

Soft skills:

Interpersonal and leadership skills (teamwork):
Written and oral communication:
Self-management and work habits (time management):
Research and information management:
Project management:
Analysis and Problem-solving abilities:

Language skills:

German (Native language)
English (Fluent in spoken and written)
French (Basic knowledge)

Computer literacy:

Microsoft Office 10
Computational tools to analyze and represent scientific data

Scientific Skills:

Molecular biological techniques (e.g. plasmid cloning, sequencing, electroporation, transfection)
Microbiological techniques (e.g. bacterial transformation)
Virologic techniques (e.g. production of recombinant viruses and pseudotyped virus particles, infection experiments (BSL3))
Immunological techniques (e.g. ELISA, Western Blot)
Flow cytometry (BD FACS Canto II, LSR Fortessa)
Cell culture
Routine analytics

Practical experiences

2015-2016 Scientific assistant University hospital Freiburg i.Br., Germany. Group Thimme/Hofmann.

2013-2015 Scientific assistant, IMTEK, Albert-Ludwigs University, Freiburg i.Br., Germany. Group Kerzenmacher.

Education

Higher education

2016-2019 PhD student INSERM U1110, Institute of Viral and Liver Disease, Strasbourg, France. Thesis title: "Impact of O-linked N-acetylglucosamine transferase on late steps of the hepatitis C replication cycle" (Defense scheduled: October 29th 2019).

2014-2016 Master of Science Biology (Immunology, Virology), Albert-Ludwigs-University, Freiburg i.Br., Germany. Thesis title: "TCF1 expression in human virus-specific CD8⁺ T cells" (degree: 1.4).

2009-2014 Bachelor of Science Biology, Albert-Ludwigs-University, Freiburg i.Br., Germany. Thesis title: "Conjugative transfer experiments with Corynebacteria"(degree: 1.2).

Secondary education

2000-2009 General qualification for university entrance, Scheffel-Gymnasium, Lahr/Schwarzwald, Germany (degree: 2.5).

Impact of OGT on late steps of the hepatitis C virus replication cycle

Hepatitis C is caused by the hepatitis C virus (HCV) leading in most subjects to chronic liver infection resulting in chronic hepatitis and progressive liver disease and thereby to development of lethal complications, i.e. cirrhosis and hepatocellular carcinoma (HCC). Infection of human hepatocytes by HCV is a multistep process involving viral and host factors. microRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate gene expression. A functional high-throughput miRNA mimic screen identified miR-501-3p and miR-619-3p as novel modulators of HCV assembly/release. We discovered that these miRNAs regulate O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) protein expression and identified OGT and O-GlcNAcylation as regulators of HCV morphogenesis and infectivity. Furthermore, increased OGT expression in patient-derived liver tissue was associated with HCV liver disease and cancer. In addition to its effect on HCV morphogenesis, OGT may thus play a role in HCV-induced liver disease and hepatocarcinogenesis.

Keywords: Hepatitis C virus, microRNA, OGT, hepatocellular carcinoma

L'hépatite C est causée par le virus de l'hépatite C (HCV) qui est responsable de maladies chroniques du foie et l'une des principales causes de développement du carcinome hépatocellulaire (CHC). L'infection des hépatocytes humains par le HCV est un processus en plusieurs étapes impliquant des facteurs viraux et des facteurs de l'hôte. Les microARN (miR) sont de petits ARN non codants qui régulent l'expression des gènes au niveau post-transcriptionnel. En utilisant un criblage génomique de miR, nous avons identifié miR-501-3p et miR-619-3p comme modulateurs de l'assemblage et l'export du HCV. Nous avons découvert que ces miR régulent l'expression de l'OGT (UDP-N-acétylglucosamine-peptide N-acétylglucosaminyltransférase) et identifié l'OGT et la O-GlcNAcylation comme régulateurs de la morphogénèse et de l'infectiosité du HCV. De plus, l'expression de l'OGT dans les tissus hépatiques de patients infectés par le HCV était associée à la maladie hépatique et au cancer. En plus de son effet sur la morphogénèse du HCV, l'OGT peut donc jouer un rôle dans les maladies hépatiques induites par le HCV et l'hépatocarcinogénèse.

Mots-clés : virus de l'hépatite C, microARN, l'OGT, carcinome hépatocellulaire