Hepatitis C virus entry – molecular mechanisms and antiviral targets

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HEPCam
Cambridge, June 27, 2013
HCV entry – a key step within the viral life cycle

- First step of virus-host interactions
- Required for initiation, spread and maintenance of infection
- Mediated by viral envelope glycoproteins and host factors
- Target of first line host immune responses – neutralizing antibodies
- Important for pathogenesis of HCV infection and liver disease
- Important target for antiviral strategies
Milestones: Development of cell culture model systems for the study of HCV life cycle


HCV entry: host factors

- Highly sulfated heparan sulfate (hsHS)
- Scavenger receptor class B type I
- Tetraspanin CD81
- Claudin-1 Occludin

Barth et al. J. Biol. Chem. 2003
Scarselli et al. EMBO J. 2002
Pileri et al. Science 1998
Concepts in molecular virology of HCV entry

- Virus-associated lipoproteins and cholesterol are relevant for HCV entry
- Cell entry is regulated by host cell kinases
Virion-associated lipoproteins and HCV entry


- Enveloped subviral particles containing apoB (Scholtes et al. Hepatology 2012)


- ApoE-syndecan interactions mediate viral entry (Shi et al. J. Virol. 2012; Lefèvre et al. EASL 2013)

- HCV-apoE-lipid interactions mediate viral evasion from host neutralizing responses (Felmlee et al. EASL 2013)
Niemann-Pick C1-like cholesterol absorption receptor (NPC1L1) is a co-factor for HCV entry

- NPC1L1 - cholesterol-sensing receptor, cholesterol absorption and whole-body cholesterol homeostasis
- Re-absorbs cholesterol from bile
- Role of NPC1L1 in HCV entry shown by siRNA and antibody studies
- NPC1L1 inhibitor Ezetimibe inhibits HCV infection in vitro and in vivo
- NPC1L1-mediated HCV entry appears cholesterol-dependent and occurs postbinding
- Potential indirect effect by altering membrane cholesterol content

Cartoon from Lupberger / Baumert J. Hepatol. 2012

Concepts in molecular virology of HCV entry

- Virus-associated lipoproteins and cholesterol are relevant for HCV entry
- Cell entry is regulated by host cell kinases
Identification of host kinases required for HCV entry and infection using a functional siRNA screen

siRNA library targeting 691 cellular kinases and associated proteins (pool of four siRNA)

Silencing of kinase expression in Huh7 cells by transfection of target-specific siRNAs

72 h

Infection of Huh7 with HCV pseudoparticles (HCVpp) and recombinant cell culture derived HCV (HCVcc)

48 h

Analysis of viral infection by luciferase reporter gene expression

Validation using individual siRNA

Data analysis using statistical modeling (limma)
Functional genomics for target discovery: siRNA screen identifies HCV entry factors as antiviral targets

- Functional siRNA HCV entry screen in Huh7 liver-derived cells
- Identification of a functional network of host cell kinases as HCV co-factors
- Among these kinases was EGFR, a well characterized drug target in cancer therapy
- Identification of a novel class of antivirals
- Investigator-initiated clinical trial

Lupberger and Zeisel et al. Nature Medicine 2011
EGFR expression and function are relevant for HCV entry

- Silencing of EGFR expression inhibits HCV entry in human hepatocytes and viral entry is rescued by exogenous EGFR expression

- Clinical EGFR inhibitor Erlotinib inhibits dose-dependently HCV entry and infection

- EGF increases HCV entry and Erlotinib reverses this increase

Lupberger*, Zeisel* et al., Nat. Med. 2011
Molecular mechanism: EGFR/HRas signaling promotes formation of the HCV host cell receptor complex

- HCV entry requires HRas activation downstream of receptor tyrosine kinase signaling
- HRas associates with membrane microdomains containing entry factors CD81 and claudin-1
- CD81-associated proteins Rap2B and integrin beta1 are additional HCV entry co-factors
- HRas triggers lateral membrane diffusion of CD81 and host entry factor complex formation
Model of HCV entry

Adapted from Gerold & Rice,
Clinical impact of HCV entry: Liver transplantation

- HCV-related cirrhosis and hepatocellular carcinoma

  Major indications for liver transplantation (LTx)

- Universal re-infection of the graft
- Absent strategy for prevention of re-infection
- Accelerated progression of disease to cirrhosis
- Low efficacy and poor tolerance of antiviral therapy
- Recurrent liver disease with poor outcome
HCV re-infection after liver transplantation

- Early - immediately following transplantation
- Rapid - viral spread within days following engraftment
- Efficient - high viral load despite presence of antibodies
- « Genetic bottleneck »

Plasma 7 days after LT

Cloning and sequencing E1-E2
25 clones / time point / patient

Identification of selected and non selected variants

Plasma Before LT

Methods: study of HCV entry and neutralization using the retroviral HCV pseudotype model system

Enhanced viral entry and escape from neutralization a key determinants for selection of HCV variants during liver transplantation

- Evolution of viral quasispecies changes following transplantation
- Variants re-infecting the liver graft are characterized
  - most efficient viral entry
  - poor neutralization by patient antibodies
- Genetic variability allows the virus to rapidly adapt, infect the graft and evade neutralizing responses
- Viral entry is a target for prevention and therapy of liver graft infection

Fafi-Kremer / Baumert J. Exp. Med. 2010
Fofana / Baumert Gastroenterology 2012
Mapping of mutations mediating enhanced entry and escape from neutralization

Transplant Patient

Before LT

After LT

E1 E2

HVR1 HVR2 CD81 Binding Domains

Mapping of mutations mediating enhanced entry and escape from neutralization
Altered cell entry factor use determines viral evasion of escape variants

A. 
P01 VL/JFH1

B. 

C. 

D. 

Fofana et al. Gastroenterology 2012

Collaboration
R. Bartenschlager, Heidelberg
Viral entry and escape from neutralization are key determinants of HCV liver graft infection (Fafi-Kremer et al., J Exp Med 2010)

Enhanced entry and neutralization escape through altered cell host receptor use (Fofana, Fafi-Kremer et al., Gastroenterology 2012)

A novel and clinically important mechanism of viral evasion: co-evolution between receptor usage and escape from neutralization

Viral entry is a promising target for the development of antiviral strategies
Host entry factors as targets for antiviral therapy

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<td>Mouse model</td>
<td>Syder et al. J. Hepatol. 2011, RCT ongoing</td>
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<td>Vanwolleghem et al. Hepatology 2008</td>
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<td>Matsumura et al. Gastroenterology 2009</td>
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Adapted from Zeisel MB, Fofana I, Fafi-Kremer S, Baumert TF J. Hepatol. 2011
Inhibition of HCV infection using antibodies targeting cell entry factor Claudin-1 (CLDN1)

HCV entry via CD81/CLDN1 co-receptor complex

CLDN1-specific antibody blocks viral infection

Anti-CLDN1 antibodies inhibit infection of HCVpp bearing envelope glycoproteins in a pangenotypic manner

Huh7.5.1 cells

Genotype 1a
Genotype 1b
Genotype 2a
Genotype 3a
Genotype 4
Genotype 5
Genotype 6
VSV

% HCV/pp infection

mAb (μg/ml)

CTRL IgG
OM-6E1-B5
OM-8A9-A3
OM-6D9-A6
OM-7C8-A8
OM-4A4-D4
OM-7D3-B3

Fofana et al. Gastroenterology 2010
Entry inhibitors inhibit infection of HCV escape variants that are resistant to host neutralizing antibodies.

A. Human Hepatocytes + Anti-CLDN1 mAb

Transplant-derived escape variants HCVpp

Method: Pestka et al. PNAS 2007, Fafi-Kremer et al. JEM 2010

B. Anti-HCV autologous serum Anti-CLDN1 mAb

% HCVpp entry

HCV-J

CTRL serum autologous anti-HCV transplant serum

% HCVpp entry

CTRL anti-CLDN1 mAb (OM-7D3-B3) mAb

P02VH P02VI P02VJ P04VC P04VD P05VF P06VG P06VI

P02VH P02VI P02VJ P04VC P04VD P05VF P06VG P06VI

P04VC P04VD P05VF P06VG P06VI

P04VC P04VD P05VF P06VG P06VI

P06VG P06VI

P06VG P06VI
Synergistic effect of entry inhibitors and DAAs on inhibition of HCV infection

A. daclatasvir + anti-CLDN1 mAb

B. sofosbuvir + erlotinib

Fofana I*, Xiao F* et al. EASL 2013
Combination of DAA with HTEIs prevent antiviral resistance and result in sustained viral clearance in cell culture models.

Graph showing the HCV load (log_{10} copies/ml) over time post-treatment (days) for different groups:
- CTRL mAb
- anti-CLDN1
- simeprevir
- simeprevir + anti-CLDN1

*Confirmed by Abbott qPCR
Small animal models for HCV infection


- Transgenic mice expressing HCV entry factors – partial life cycle, immunocompetent (Dorner et al. Nature 2011)
Human chimeric mouse liver architecture is similar to human liver

**Chimeric human mouse liver**

- **uninfected**
- **HCV infected**

**Human liver**

- **huCD10**
- **huCLDN1**

Collaboration: Jane A. McKeating, University of Birmingham
CLDN1-specific mAb completely prevents of HCV infection in human liver chimeric mice

Mailly et al. 2013, submitted
Clearance of persistent HCV infection using a CLDN1-specific antibodies in human liver chimeric mice

A. Viral load

B. Genotype 2a

Genotype 1b

Mailly et al. 2013, submitted
Absence of detectable antiviral resistance in HCV infected mice

A.

Inoculum (HCV Jc1) → Anti-CLDN1 mAb → Post-treatment serum

B.

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C.

Viral pp entry in the presence of anti-CLDN1 mAb (% of control mAb)
Mechanism of action 1: anti-CLDN1 mAb dose-dependently inhibits HCV cell-cell transmission and viral spread

A. Cell-cell transmission assay

- GFP⁺ HCV⁺ target cells
- GFP⁺ HCV⁺ producer cells
- + anti-HCV E2 prevents cell-free transmission
- Co-cultivation of producer and target cells in presence of anti-HCV E2


B. Anti-CLDN1 inhibit cell-cell transmission

Infected target cells (%)

Control mAb
Anti-CLDN1 (µg/mL)

0.1 1 10 100

Post-infection time (day)

Luc-Jc1 infection

HCVcc infection (Log_{10} RLU)

Anti-CLDN1 mAb

Control mAb

0 0.1 1 10 100

F. Xiao, unpublished 2013
Mechanism of action 2: anti-CLDN1 modulates virus-induced signaling in HCV infected cells

- MAPK signaling is induced in HCV-infected cells
- Anti-CLDN1 inhibits ERK1/2 phosphorylation suggesting interference with virus-induced signaling

J. Lupberger, unpublished 2013
Unraveling the mechanisms of HCV entry identifies a novel class of antivirals for prevention of liver graft infection

- Entry inhibitors - early stage of development (preclinical, early clinical)
- Proof-of-concept for prevention and treatment of HCV infection
- Pan-genotypic efficacy, effective against escape variants and resistant virus
- Efficient and simple antiviral strategy for prevention of liver graft infection
- Prevention/treatment of antiviral resistance in chronic infection
Acknowledgements

Inserm Unit 1110, Laboratory of Excellence HepSys University of Strasbourg, France
Joachim Lupberger
Laetitia Zona
Isabel Fofana
Dan Felmlee
Fei Xiao
Mathieu Lefèvre
Catherine Fauvelle
Mohammed-Lamine Hafirassou
Nauman Zahid
Marine Turek
Marie Parnot
Christine Thumann
Laurent Mailly
Quentin Lepellier
Samira Fafi-Kremer
Françoise Stoll-Keller
Michel Doffoël
François Habersetzer
Patrick Pessaux
Heidi Barth
Mirjam B. Zeisel
Catherine Schuster

Molecular Virology, U Heidelberg, Germany
Marie-Sophie Huet, Gang Long, Ralf Bartenschlager

Inserm U758, Human Virology, ENS Lyon, France
Els Verhoeyen, Dimitri Lavillette, François-Loïc Cosset

Department of Medicine, University Hospital Hamburg
Marc Lutgehetmann, Maura Dandri

Division of Immunity and Infection, U Birmingham, UK
Garrick K. Wilson, Jane A. McKeating

Twincore, Medizinische Hochschule Hannover, Germany
S. Haid, E. Steinmann, T. Pietschmann

Center for Vaccinology, Ghent University, Ghent, Belgium
Philip Meuleman, Geert Leroux-Roels

Institute of Virology, University Hospital Essen, Germany
Michael Roggendorf

Dept. of Biomedicine, Hepatology, University of Basel, CH
François Duong, Markus Heim

The Rockefeller University, New York, NY, USA
Charles M. Rice