The Bystander Effect in Hepatitis C Virus Infection: Cellular Interactions Between Infected Cells and Uninfected Cells

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Abstract

Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide that often results in progressive liver disease in the form of fibrosis, cirrhosis and in some cases, hepatocellular carcinoma. The mechanisms responsible for progression to advanced liver disease are poorly understood, but this primarily occurs as a result of chronic hepatic inflammation. Despite universal involvement of the liver in this inflammatory and fibrogenic process, only a small percentage of hepatocytes are infected. We therefore hypothesised that the pathological effect of the virus is extended beyond the infected hepatocyte to uninfected 'bystander' cells by cellular interactions between these cells. To study this hypothesis, we developed *in vitro* cell culture model systems to observe the interactions between HCV-infected and uninfected Huh-7 cells and stellate cells.

HCV permissive Huh-7 cells are relatively unresponsive to virus infection with regard to the innate immune response. This is due to a lack of expression of the pattern recognition receptor Toll-like receptor 3 (TLR3), which is known to play an important role in the innate immune response to HCV infection. To restore the response of infected Huh-7 cells to HCV we generated a Huh-7 cell line stably expressing functional TLR3. We subsequently demonstrated by microarray analysis upregulation of TLR3 response genes such as chemokines and classical interferon response genes (ISGs) in response to HCV infection of these cells.

To prevent HCV infection of Huh-7 'bystander' cells we also generated a line refractory to HCV infection by shRNA knockdown of the essential HCV entry

receptor CD81. This cell line was also tagged with GFP to allow for FACS sorting of uninfected cells in co-culture.

We subsequently employed these cell lines in conditioned media and co-culture model systems to examine the cell interactions mediated by soluble factors and cell-to-cell contact at the level of the transcriptome using Affymetrix microarray analysis. Although the effect of HCV-infected hepatocytes on uninfected 'bystander' hepatocytes was not dramatic, preliminary data suggested that suppressor of cytokine signalling 3 (SOCS3), a known inhibitor of endogenous interferon signalling pathways, is upregulated in uninfected Huh-7 cells co-cultured with HCV-infected TLR3-positive Huh-7 cells. Furthermore we also demonstrated that HCV-infected cells exert an antiviral effect on other infected cells, possibly via exosome-mediated signalling, and can increase expression of pro-fibrogenic markers in hepatic stellate cells. We also showed that TLR3-positive uninfected Huh-7 cells enhance chemokine expression in HCV-infected hepatocytes.

In summary, we have generated stable cell lines that can be employed in an *in vitro* cell culture model system to study the interactions between HCV-infected hepatocytes and other resident liver cells such as uninfected hepatocytes and hepatic stellate cells. We have demonstrated bidirectional cross-talk between cell types, and the observed exerted effects are likely to contribute to the pathogenesis of chronic liver disease in HCV infection by recruiting uninfected cells into the pro-inflammatory and pro-fibrogenic response to HCV infection. The knowledge gained from this work contributes to our understanding of the mechanisms underlying progression of liver disease in HCV infection.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

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Presentations, Publications and Awards

Presentations

HCV-induced changes in gene expression in non-infected ‘bystander’ Huh-7 cells. 18th International Symposium on Hepatitis C and Related Viruses, Seattle, USA, September 2011 (poster).

HCV-induced changes in gene expression in non-infected ‘bystander’ Huh-7 cells. ACH2 Workshop, Adelaide, Australia, June 2012 (oral).

HCV-induced changes in gene expression in non-infected ‘bystander’ Huh-7 cells. HCV 2012: 19th International Symposium on Hepatitis C and Related Viruses, Venice, Italy, October 2012 (poster).

The effect of hepatitis C infected Huh-7 cells on 'bystander' cells. Australian Gastrointestinal Week, Melbourne, Australia, October 2013 (poster of merit).

The effect of hepatitis C infected Huh-7 cells on 'bystander' cells. HCV 2013: 20th International Symposium on Hepatitis C and Related Viruses, Melbourne, Australia, October 2013 (poster).

Publications

TLR3-dependent cross-talk between HCV-infected and uninfected hepatocytes. Muller, K.R., Eyre, N.S., Van der Hoek, K.H., Li, K., Beard M.R. (in preparation).

Awards

MSD Hepatology Young Achiever Award, 2012

Abbreviations used

ATP	adenosine triphosphate
bp	base pairs
BSA	bovine serum albumin
°C	degrees Celsius
CCL	chemokine (C-C motif) ligand
cDNA	complementary DNA
CLDN	claudin
cm	centimetres
CMV	cytomegalovirus
COL1a1	collagen type 1 alpha 1
C_T	threshold cycle
CXCL	chemokine (C-X-C motif) ligand
CXCR	chemokine (C-X-C motif) receptor
Da	daltons
DAPI	4',6-diamidino-2-phenylindole
dATP	deoxyadenosine-5’-triphosphate
dCTP	deoxycytosine-5’-triphosphate
DDIT4	DNA damage inducible transcript 4
DDX60	DEAD (Asp-Glu-Ala-Asp) Box Polypeptide 60
dGTP	deoxyguanosine-5’-triphosphate
dH_2O	deionised water
DMEM	Dulbecco’s Modified Eagle Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate

dsRNA	double stranded RNA
dTTP	deoxythymidine-5’-triphosphate
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EMCV	encephalomyocarditis virus
ER	endoplasmic reticulum
FACS	fluorescence-activated cell sorting
FBS	foetal bovine serum
ffu	focus-forming units
g	grams
GAGs	glycosaminoglycans
GFP	green fluorescent protein
HABP2	hyaluronic acid binding protein 2
HCV	hepatitis C virus
HCVcc	cell-culture propagated hepatitis C virus
HCC	hepatocellular carcinoma
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	human immunodeficiency virus
HRP	horseradish peroxidase
IFI6	interferon alpha-inducible protein 6
IFIT	interferon-induced protein with tetratricopeptide repeats
IFITM	interferon induced transmembrane protein
IFN-α	interferon alpha
IFN-β	interferon beta
IL	interleukin
IP-10	interferon gamma-induced protein 10
IRES	internal ribosome entry site

IRF	interferon regulatory factor
ISG	interferon stimulated gene
ISGF3	interferon-stimulated gene factor-3
ISRE	interferon-stimulated response element
JAK	janus kinase
kb	kilobases
kDa	kilo Daltons
kV	kilovolts
LDL	low-density lipoprotein
LDL-R	low-density lipoprotein receptor
µF	microfarad
µg	micrograms
µl	microlitres
µM	micromolar
mA	milliamps
MAVS	mitochondrial antiviral-signalling protein
MCP1	monocyte chemotactic protein-1
mg	milligrams
MIG	monokine induced by gamma interferon
MIP1β	macrophage inflammatory protein-1β
ml	millilitres
mM	millimolar
MOI	multiplicity of infection
mRNA	messenger RNA
MSR1	class A scavenger receptor type 1
MW	molecular weight
NCR	non-coding region
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells

ng	nanograms
nm	nanometres
nM	nanomolar
NK	natural killer
NS	non-structural
OAS	2'-5'-oligoadenylate synthetase
OCLN	occludin
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PAMP	pathogen-associated molecular pattern
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
pg	picograms
pM	picomolar
pmol	picomoles
poly I:C	polyinosinic:polycytidylic acid
PKR	protein kinase R
PRR	pattern recognition receptor
qPCR	quantitative polymerase chain reaction
qRT-PCR	real-time reverse-transcription PCR
RANTES	Regulated on Activation, Normal T cell Expressed and Secreted
RdRp	RNA-dependent RNA polymerase
RELN	reelin
RIG-I	retinoic acid-inducible gene I
RIPA	radio-immunoprecipitation assay
RNA	ribonucleic acid

ROS	reactive oxygen species
rpm	revolutions per minute
SCID	severe combined immunodeficiency
SDS	sodium dodecyl sulfate
shRNA	short hairpin RNA
siRNA	small interfering RNA
SOC	super optimal broth with catabolite repression
SOCS3	suppressor of cytokine signalling 3
SPP	secreted phosphoprotein 1
SR-B1	scavenger receptor class B1
STAT	signal transducer and activator of transcription
SV40	simian virus 40
SVR	sustained virological response
TAE	Tris-Acetic Acid-EDTA
TARC	thymus and activation-regulated chemokine
TBS-T	Tris-buffered saline-Tween 20
TEMED	N,N,N’,N’-tetramethylethylenediamine
TfRTCA	transferrin receptor-truncated amino terminus
TGF-β	transforming growth factor beta
TIMP-1	tissue inhibitor of metalloproteinase 1
TIR	toll IL-1 receptor
TLR3	toll-like receptor 3
TNF	tumour necrosis factor
TRIF	toll-interleukin-1 receptor domain-containing adaptor inducing IFN-β
Tris	3,3,5,5-tetramethylbenzidine
U	units
uPA	urokinase-type plasminogen activator
UTR	untranslated region

UV	ultraviolet
V	volts
VLDL	very low-density lipoprotein
VSV	vesicular stomatitis virus
v/v	volume per volume
w/v	weight per volume
x g	G-force

Materials Providers

Addgene	Massachusetts, USA
Affymetrix	California, USA
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AppliChem	Darmstadt, Germany
Beckman Coulter	Miami, FL, USA
Becton Dickinson	New Jersey, USA
Bioline	Sydney, Australia
BioRad Laboratories	California, USA
Biotium	California, USA
Biovision	California, USA
Clontech	California, USA
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