Defining and Characterising the anti-HCV Actions of the Interferon-Induced Transmembrane proteins

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Abstract

Hepatitis C virus (HCV) is a significant human pathogen of the liver that in the majority of infected individuals causes a chronic infection of the liver. This may over time culminate in the development of severe liver disease such as cirrhosis and hepatocellular carcinoma. Prior to 2012, the only treatment option available for HCV infection was combination therapy with pegylated interferon-α (IFN-α) and ribavirin. However, the recent development and addition of direct acting antivirals (DAAs) into treatment regimes has significantly improved sustained virological response rates. While the ultimate aim is for IFN-α free therapy, IFN-α is often required in combination with the DAAs to reduce the development of viral resistance and due to cost. It is clear that IFN (either exogenous or endogenous) can induce an antiviral state in HCV infected cells; however, the exact mechanisms that underpin this action remain unclear.

The interferon-induced transmembrane (IFITM) family of proteins - IFITM1, IFITM2 and IFITM3 has recently been identified as important host effector molecules of the type I IFN response against a broad range of RNA viruses. During the course of this PhD study, a number of investigations identified the IFITM proteins to be potent antiviral effectors against HCV; however, the mechanism(s) for this antiviral activity remains contradictory. In this thesis, we demonstrate that IFITM1, IFITM2 and IFITM3 play an integral role in the IFN response against HCV and act specifically to inhibit early and late stages of HCV entry to inhibit infection. We reveal that IFITM1 localises to the cell surface in hepatocytes and interacts with the host entry factor CD81 to limit HCV entry. Furthermore, the N-terminus, in particular amino acids 21-28, of

IFITM1 plays an important role in this anti-HCV activity, while the C-terminus is found to be important for localisation to the cell surface.

We also established that in hepatocytes, IFITM2 and IFITM3 localise to the late and early endosomes respectively, as well as the lysosome, indicating that IFITM2 and IFITM3 follow the established paradigm of targeting the late entry stages of HCV infection. Furthermore, we have demonstrated that S-palmitoylation of all three IFITM proteins is essential for both anti-HCV activity and cellular localisation, while the conserved tyrosine residue in the N-terminus of IFITM2 and IFITM3 plays a significant role in protein localisation. However, this tyrosine was found to be dispensable for anti-HCV activity, with mutation of the tyrosine resulting in an IFITM1-like phenotype with the retention of anti-HCV activity and co-localisation of IFITM2 and IFITM3 with CD81.

In conclusion, we propose that the IFITM proteins act in a coordinated manner to restrict HCV infection by targeting the endocytosed HCV virion for lysosomal degradation and demonstrate that the actions of the IFITM proteins are indeed virus and cell-type specific. We believe we have significantly added to our understanding of the interplay between HCV and the host innate immune response and that in the long term these findings will aid in the generation of novel and targeted anti-HCV therapeutics for patients chronically infected with HCV.

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, in the future, will be used in a submission in my name for any other degree or diploma in any other university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Publications Arising from this PhD

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Awards Received During PhD

2012 School of Molecular and Biomedical Science School Symposium Poster Prize – 2nd Place - $200

2012 Nomination by the Golden Key International Honour Society to attend the 2013 International Scholar Laureate Program (ISLP) Delegation on Medicine

2012 Australian Centre for Hepatitis Virology Travel Award for HCV Research – $2500
National award - one awarded per year for travel to international HCV meeting.

2011 Dawes Top-Up Scholarship, Royal Adelaide Hospital Research Fund Grant Round – funded from 2012 to 2014

Presentations Arising From PhD

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Narayana, S.K., Helbig, K.J., McCartney, E.M. Eyre, N.S., Aloia, A.L. and Beard, M.R. The interferon-induced transmembrane protein (IFITM) family exhibit anti-HCV activity. 20th International Symposium on Hepatitis C Virus and Related Viruses, Melbourne, Australia, 2013. (poster presentation)

Narayana, S.K., Helbig, K.J., Eyre, N.S., McCartney, E.M. and Beard, M.R. The interferon-induced transmembrane protein (IFITM) family exhibit anti-HCV activity. 19th International Symposium on Hepatitis C Virus and Related Viruses, Venice, Italy, 2012. (poster presentation)

K.J. Helbig, J.M. Carr, **S.K. Narayana**, E.M. McCartney, N.S. Eyre and M.R. Beard. The ISG viperin has novel anti-hepatitis C virus activity through interaction with a

HCV pro-host cell factor. Keystone Symposia 2012 - Innate Immunity, Colorado, USA, 2012. (poster presentation)

McCartney E.M., Helbig, K.J., Eyre, N.S, **Narayana, S.K**. and Beard, M.R. The role of STAT3 in the life cycle of HCV. 18th International Symposium on Hepatitis C Virus and Related Viruses, Seattle, USA, 2011. (poster presentation)

NATIONAL

Narayana, S.K., Helbig, K.J., McCartney, E.M. Eyre, N.S., Aloia, A.L. and Beard, M.R. The interferon-induced transmembrane protein (IFITM) family exhibit anti-HCV activity. Infection and Immunity Conference, Lorne, Australia, 2014. (poster presentation)

Narayana, S.K., Eyre, N.S., McCartney, E.M., Van der Hoek, K., Helbig, K.J., and Beard, M.R. The interferon-induced transmembrane (IFITM) proteins have anti-HCV activity *in vitro*. Australian Centre for Hepatitis Virology workshop, Adelaide, Australia, 2012. (oral presentation)

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Beard M.R., **Narayana, S.K.**, Eyre, N.S., Yip, E.Y., Lemon, S.M. and Helbig, K.J. The ISGs viperin and the IFITM family have novel anti-Hepatitis C Virus activity. Infection and Immunity Conference, Lorne, Australia, 2011. (oral presentation)

Narayana S.K., Eyre N.S., Van der Hoek, K., Helbig K.J., and Beard, M.R. Identification of novel interferon stimulated genes (ISGs) that control HCV *in vitro*. Australian Centre for Hepatitis Virology workshop, Yarra Valley, Australia, 2010. (oral presentation)

Materials Providers

Abcam	Cambridge, UK
Ambion	Texas, USA
Amersham Pharmacia Biotech	Birminghamshire, UK
Amrad Biotech	Boronia, VIC, Australia
Anogen	Ontario, Canada
Applied Biosystems	Warrington, UK
Becton Dickson Labware	New Jersey, USA
Biomol	New Jersey, USA
BioRad Laboratories	California, USA
Cell Signaling	Massachusetts, USA
Chemicon International	Massachusetts, USA
Cohu	California, USA
DAKO	California, USA
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SPSS Inc	Illinois, USA
Stratagene	California, USA
UVP Inc	California, USA
Vector Laboratories	California, USA
Vision Systems	Mount Waverley, VIC, Australia

Abbreviations Used

A	adenosine
aa	amino acids
bp	base pairs
BSA	bovine serum albumin
BVDV	bovine viral diarrhoea virus
C	cytosine
° C	degrees Celsius
cDNA	complimentary deoxyribosenucleic acid
CLDN1	claudin-1
CHC	chronic hepatitis C
CIL	cytoplasmic intracellular loop
CMV	cytomegalovirus
dATP	deoxyadenosine-5’-triphosphate
dCTP	deoxycytosine-5’-tripshosphate
DEPC	diethyl pyrocarbonate
DAA	direct acting antiviral
dGTP	deoxyguanosine-5’-triphosphate
dH_2O	deionised water
DMEM	Dulbecco’s Modified Eagle Medium with HEPES
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
dTTP	deoxythymidine-5’-triphosphate
DENV	Dengue virus
EBoV	Ebola virus
EDTA	ethylene diamine tetra acetic acid
ER	endoplasmic reticulum

FCS	foetal calf serum
FITC	fluorescein isothiocyanate
FFU	focus forming unit
g	grams
G	guanosine
GAPDH	glyceraldehyde-3-phosphate deydrogenase
GAS	gamma-activated sequence
GFP	green fluorescent protein
HCC	hepatocellular carcinoma
HBV	hepatitis B virus
HCV	hepatitis C virus
hr	hour(s)
HRP	horse radish peroxidase
HIV	human immunodeficiency virus°°°°
HPV	human pappilomavirus
IAV	influenza virus
IDU	infecting drug use
IFITM	interferon-induced transmembrane protein
IFN-α	interferon alpha
IFN-β	interferon beta
IFN-γ	interferon gamma
IFN-λ	interferon lambda
IRES	internal ribosome entry site
ISRE	interferon stimulated response element
JAK	janus kinase
kb	kilobase
kDa	kilo Dalton
L-Agar	LB + agar
LD	lipid droplet

LB	Luria Bertani broth
LDL	low density lipoproteins
Luc	luciferase
µg	micrograms
µl	microlitres
µM	micromolar
mA	milliamps
mg	milligrams
ml	millilitres
mM	millimolar
MCS	Multiple Cloning Site
MEM	Minimum Essential Medium
min	minute(s)
mRNA	messenger RNA
MW	molecular weight
MLV	murine leukemia virus
ng	nanograms
nM	nanomolar
N/A	not applicable
nt	nucleotide
NTD	N-terminal domain
NF-κB	nuclear factor-kappa-light chain enhancer
OCLN	occludin
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline; 0.15M NaCl, 6M K_2HPO_4, 2mM KH_2PO_4 (pH 7)
PAMP	pathogen associated membrane patterns

PCR	polymerase chain reaction
PHH	primary human hepatocyte
PRR	pathogen recognition receptors
PTM	post-translational modification
pg	picograms
pmol	picomolar
RNA	ribonucleic acid
rpm	revolutions per minute
RFP	red fluorescent protein
RdRp	RNA-dependent-RNA polymerase
RC	replication complex
RIG-I	retinoic acid inducible gene I
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
sd	standard deviation
SDS	sodium dodecyl sulfate
sec	second(s)
SEM	standard error of mean
ss	single stranded
siRNA	small interefering RNA
SR-BI	scavenger receptor class B member I
STAT	signal transducer and activator of transcription
SVR	sustained virological response
T	thymidine
TAE	0.04M Tris (pH 8), 0.04M Acetic Acid, 1mM EDTA
TEMED	N,N,N’,N’-tetramethylethyethylenediamine

Tris	3,3,5,5-tetramethylbenzidine
TLR	Toll-like receptor
TYK2	tyrosine kinase 2
U	units
UTR	untranslated region
V	volts
VSV	vesicular stomatis virus
w/v	weight per volume
WNV	West Nile Virus