Molecular interactions between alcohol, hepatitis C virus and interferon

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Discipline of Microbiology and Immunology

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THE UNIVERSITY of ADELAIDE

A dissertation submitted to The University of Adelaide

In candidature for the degree of

Doctor of Philosophy in the Faculty of Science

May 2011

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Abstract

Hepatitis C virus (HCV) is a significant human pathogen that in many cases, establishes a chronic life long infection of the liver, resulting in progressive liver disease that culminates in the development of cirrhosis and hepatocellular carcinoma (HCC). The only treatment option available for HCV infection is a combination therapy of Interferon-α (IFN-α) and Ribavirin. However, it is only successful in a limited number of patients. There are a number of co-factors that accelerate liver disease in chronic hepatitis C (CHC) and one of the most significant factors is alcohol consumption. Furthermore, alcohol consumption has been shown to reduce the efficacy of IFN-α treatment. Despite these clinical observations, the molecular mechanisms by which alcohol exerts these effects are unknown and remain relatively unexplored. This is largely due to the lack of an appropriate model system to enable studies into the interaction between the HCV life cycle, alcohol metabolism and IFN. To overcome this limitation, we have developed an *in vitro* cell culture model system that enables Huh-7 cells to metabolise alcohol (ethanol), via the enzyme cytochrome P4502E1 (CYP2E1), while also supporting HCV replication directed from both the HCV replicon and infectious HCV model systems. As such, this model system has been used in this thesis to extensively investigate the interactions between alcohol metabolism, HCV and IFN.

It is known clinically that HCV infected persons who consume alcohol, have exacerbated liver disease and in some cases increased serum of HCV. One postulated mechanism for this effect is that alcohol consumption increases HCV replication, which in turn leads to increased viral burden in the liver and associated pathogenic effects. We have shown that CYP2E1 mediated metabolism of alcohol increases HCV RNA replication *in vitro*, in both the replicon and infectious HCV model systems. Furthermore, we have demonstrated that this process is mediated via the oxidative

stress produced by alcohol metabolism, as the anti-oxidant NAC blocked this alcohol-induced increase in HCV RNA replication. These observations correlate with what is noted clinically and suggest a potential mechanism whereby alcohol consumption in chronically infected HCV individuals, leads to accelerated rates of liver disease progression. These findings form a rationale to clinically investigate the use of anti-oxidant therapy in CHC patients consuming alcohol.

In this thesis we present a molecular mechanism for the reduced response rates to IFN-α therapy in HCV infected individuals consuming alcohol. Specifically we have shown that alcohol metabolism attenuates the anti-HCV activity of IFN-α via perturbation of the JAK/STAT signaling cascade and subsequently decreases the expression of anti-viral ISGs, which are the effector molecules of an IFN response. Thus alcohol metabolism seems to be able to blunt the anti-viral effects of IFN and this has implications for anti-viral directed therapy and the innate immune response to HCV infection in the liver.

Also arising from this thesis was the novel observation that levels of the oxidative stress sensitive transcription factor signal transducer and activator of transcription 3 (STAT3) were increased in the context of HCV replication and alcohol metabolism. From these observations we hypothesized that STAT3 could be a potential pro-viral host factor. We have presented strong evidence in this thesis to suggest that STAT3 is working at multiple levels to assist HCV replication. Firstly, we have shown that STAT3 is activated in the presence of replicating HCV, and we believe STAT3 may be facilitating HCV replication via the production of specific STAT3 dependent genes. Secondly, we have presented significant data in this thesis to suggest that STAT3 may be assisting HCV entry into hepatocytes via the control of microtubule dynamics. These studies emphasize the need for further investigations into the role of STAT3 in the life cycle of HCV and suggest a role for therapies directed against STAT3 in patients with CHC, in order to limit disease progression. Furthermore, the

ability of HCV to activate STAT3 and the oncogenic nature of STAT3 suggest that STAT3 could be playing a mechanistic role in the development of HCC in individuals infected with HCV.

In summary we have developed an *in vitro* model system to simultaneously evaluate the impact of HCV replication, alcohol metabolism and IFN, on each other. We have shown that alcohol metabolism increases HCV replication via an oxidative stress related mechanism and that the anti-viral action of IFN is severely attenuated in the presence of alcohol metabolism. Moreover, we have also identified STAT3 as a pro-viral host factor that may exert its effect at multiple stages of the HCV life cycle. While all of the experiments in this thesis were conducted *in vitro*, the knowledge gained from this work will aid in the design of future studies to be performed when a small animal model of HCV pathogenesis becomes available. We believe we have significantly added to our understanding of the interplay between HCV and alcohol metabolism and that in the long term these findings will aid in therapeutic responses and management of patients chronically infected with HCV.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Erin Marie McCartney 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.

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Erin Marie McCartney

May 7th, 2011

Acknowledgements

I would like to thank my supervisor Michael Beard for the opportunity to do a PhD in his laboratory and for his continued assistance and mentoring throughout the years.

I offer my sincerest gratitude to Karla Helbig and Nicholas Eyre for their excellent advice, support and technical assistance during my PhD.

I am most grateful to all members of the Hepatitis C Research Laboratory both past and present. Many of whom I count as life long friends. Specifically I would like to acknowledge Lilijana Semendric, Evelyn Yip, Gorjana Radisic, Edmund Tse, Kate Muller, Sumudu Narayana and Gemma Sharp. I would also like to thank the department of Microbiology and Immunology for the opportunity to undertake a PhD.

I would like to thank my parents Kevin and Marilyn, for their continued support, encouragement and editing of this thesis. I would also like to thank my brother Patrick, for providing invaluable encouragement both at home and from afar at the many locations he has been situated. Finally, I would like to thank my partner Tom, who moved to Adelaide during the final stages of this thesis and has been the epitome of patience. I would also like to acknowledge Tom for his graphical design contributions to figures in this thesis.

Publications Arising During PhD

McCartney EM, Helbig KJ, Beard MR (2011). The role of STAT3 in hepatitis C virus life cycle. (Manuscript in preparation)

McCartney EM, Beard MR (2010). Impact of alcohol on hepatitis C virus replication and interferon signaling. World Journal of Gastroenterology. 2010 March 21; 16(11): 1337-1343. (see Appendix XII)

McCartney EM, Semendric L, Helbig KJ, Hinze S, Jones B, Weinman S and Beard MR (2008). Alcohol metabolism increases hepatitis C virus replication and attenuates the anti-viral action of interferon. J Infect Dis. 2008 Dec 15;198(12):1766-75. (see Appendix XII)

Helbig KJ, Yip E, **McCartney EM**, Eyre NS and Beard MR. A screening method for identifying disruptions in interferon signaling reveals HCV NS3/4a disrupts Stat-1 phosphorylation. Antiviral Res. 2008 March, 77:169-176.

Awards Received During PhD

2010 Royal Adelaide Hospital Clinical Project Grant
The role of STAT3 in the life cycle of Hepatitis C virus and hepatocellular carcinoma development - $15,000

2009 Australian Centre for Hepatitis and HIV Annual Meeting, Terrigal,
ACH2 Young Investigator Travel Award for oral presentation - $5000
National award - one awarded per year for travel to international HCV meeting.

2008 Adelaide University Health Sciences Travel Fellowship - $2000

2008 School of Molecular and Biomedical Science PhD student poster award - $200

2007 Australian Centre for Hepatitis and HIV Annual Meeting, Barossa Valley, ACH2 PhD Student Oral Presentation Award – $500

2006 13th International Meeting on Hepatitis C Virus and Related Viruses, Cairns, Student Travel Grant - $1500

Presentations Arising From PhD

International

McCartney EM, KJ Helbig, MR Beard. The role of STAT3 in the life cycle of HCV. 46th European Association For The Study of The Liver, Berlin, Germany, 2011. **(poster presentation)**

McCartney EM, KJ Helbig, MR Beard. Alcohol metabolism increases HCV replication in a STAT3 dependent manner. 16th International Meeting on Hepatitis C Virus and Related Viruses, Nice, France, 2009. **(poster presentation)**

McCartney EM, L Semendric, KJ Helbig, MR Beard. The role of STAT3 in the alcohol induced increase in HCV replication. 15th International Meeting on Hepatitis C Virus and Related Viruses, San Antonio, USA, 2008. **(poster presentation)**

McCartney EM, L Semendric, KJ Helbig, MR Beard. CYP2E1 metabolism of alcohol suppresses the anti-HCV action of interferon. 13th International Meeting on Hepatitis C Virus and Related Viruses, Cairns, Australia, 2006. **(oral presentation)**

National

McCartney EM, KJ Helbig, MR Beard. Role of STAT3 on HCV replication. Australian Centre for Hepatitis Virology workshop, Yarra Valley, Australia, 2010. **(oral presentation)**

McCartney EM, L Semendric, KJ Helbig, MR Beard. Role of STAT3 and oxidative stress on HCV replication. Australian Centre for Hepatitis Virology workshop, Terrigal, Australia, 2009. **(oral presentation)**

McCartney EM, L Semendric, KJ Helbig, MR Beard. Role of STAT3 and oxidative stress on HCV replication. Australian Centre for Hepatitis Virology workshop, Barrossa Valley, Australia, 2008. **(oral presentation)**

McCartney EM, L Semendric, KJ Helbig, MR Beard. CYP2E1 metabolism of alcohol suppresses the anti-HCV action of interferon. Australian Centre for Hepatitis Virology workshop, Melbourne, Australia, 2007. **(oral presentation)**

Helbig KJ, Yip E, **McCartney EM**, Eyre NS, Beard MR.A high throughput method for screening disruptions in interferon signaling reveals NS3/4a disrupts Stat-1 phoshporylation. Australian Centre for Hepatitis Virology workshop, Melbourne, Australia, 2007. **(oral presentation)**

McCartney EM, L Semendric, KJ Helbig, MR Beard. CYP2E1 metabolism of alcohol suppresses the anti-HCV action of interferon. Australian Centre for Hepatitis Virology and HIV virology interest group inaugural workshop, Terrigal, Australia, 2005. **(oral presentation)**

Materials Providers

Abcam	Cambridge, UK
Ambion	Texas, USA
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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
CHC	chronic hepatitis C
CMV	cytomegalovirus
CYP2E1	Cytochrome P450-2E1
dATP	deoxyadenosine-5'-triphosphate
dCTP	deoxycytosine-5'-tripshosphate
DEPC	diethyl pyrocarbonate
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
EDTA	ethylene diamine tetra acetic acid
ER	endoplasmic reticulum
FCS	foetal calf serum
FITC	fluorescein isothiocyanate
g	grams
G	guanosine

GAPDH	glyceraldehyde-3-phosphate deydrogenase
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HRP	horse radish peroxidase
IFN-α	interferon alpha
IFN-γ	interferon gamma
IRES	internal ribosome entry site
ISRE	interferon stimulated response element
JAK	janus kinase
kb	kilobase
kDa	kilo Dalton
L-Agar	LB + agar
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
ng	nanograms
nM	nanomolar

N/A	not applicable
nt	nucleotide
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)
PCR	polymerase chain reaction
pg	picograms
pmol	picomolar
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
sd	standard deviation
SDS	sodium dodecyl sulfate
sec	second(s)
ss	single stranded
STAT	signal transducer and activator of transcription
STMN1	Stathmin
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
TYK2	tyrosine kinase 2
U	units

UTR	untranslated region
V	volts
w/v	weight per volume