Improving treatment predictors of HCV therapy and the impact of steatosis on the hepatocyte transcriptome and anti-HCV action of interferon

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Abstract

Hepatitis C Virus (HCV) is a significant global health issue that leads to the development of chronic liver inflammation, and subsequent establishment of cirrhosis and hepatocellular carcinoma (HCC).

Previously the standard therapy of chronic hepatitis C (CHC) was pegylated interferon α (IFN-α) and ribavirin, which had poor treatment success rates and was associated with significant side effects. Risk factors that have been shown to be associated with treatment failure include excess alcohol consumption, advanced age, diabetes and obesity. Although these negative predictors of treatment outcome have been well established in clinical practice, little is known regarding the molecular mechanism(s) of treatment failure.

Obesity is another major health issue which is associated with numerous deleterious health issues, one of which being steatosis, or non-alcoholic fatty liver disease (NAFLD). NAFLD can progress to necroinflammation of the liver or non-alcoholic steatohepatitis (NASH), leading to fibrosis and development of cirrhosis. Given how common obesity is, clinicians are commonly faced with managing patients with CHC and concurrent steatosis. Understanding the molecular mechanism(s) of interferon-based treatment failure in patients with CHC with concurrent steatosis, may allow adjuvant therapy to be targeted to those with negative predictors of treatment outcome, thus resulting in an improved virological response.

In this thesis, an *in vitro* model of steatosis has been adopted to investigate the effect of lipid loading on gene expression, in particular, interferon-stimulated genes (ISGs). Two different free fatty acids, oleic acid and palmitic acid, were used to induce steatosis in the Huh-7 hepatoma cell line. In this thesis, it was shown that steatosis

was associated with a marked alteration of gene expression, some of which interestingly were classical ISGs. This was likely due to TLR2 mediated pathways, leading to subsequent downstream NF-κβ activation and gene expression.

Through induction of steatosis by oleic and palmitic acid, it was also shown that Huh-7 cells can accentuate the effect of interferon stimulation, leading to an increased ISG expression, which is believed to be secondary to the increase in STAT1 phosphorylation.

Finally the effect of steatosis-induced ISG expression on HCV replication, as well as the responsiveness to IFN-α treatment, was investigated. Surprisingly, it was found that steatosis alone led to a modest reduction of HCV replication, with reduced interferon sensitivity, leading to a reduction in HCV knockdown when IFN-α was used. It was shown that the combination of OA:PA, HCV replication and IFN-α stimulation resulted in a significant increase in CXCL8 protein production, a cytokine known to have anti-IFN modulating activity. Moreover, exogenous addition of CXCL8 to cultured cells abrogated the anti-HCV actions of IFN-α. This highlighted a potential mechanism for IFN failure in the HCV infected liver with concurrent steatosis.

In summary, the *in vitro* model of steatosis has revealed a much better understanding of the effects that free fatty acids have on gene expression in hepatocytes, as well as their relationship with HCV infection and IFN-α therapy. As reported in this thesis, new and unexpected data has been obtained, which may lead to a different way of thinking about host-virus interactions. In the future this will hopefully be translated to better treatment options for HCV infection.

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 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.

Edmund Tse

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Acknowledgements

"Success is not final, failure is not fatal: it is the courage to continue that counts."
Winston Churchill

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Publications Arising During Ph.D.

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Tse E, Helbig KJ, Van der Hoek K, McCartney EM, Van der Hoek M, George J, Beard MR. Fatty acids induce a pro-inflammatory gene expression profile in Huh-7 cells that attenuates the anti-HCV action of interferon. J Interferon Cytokine Res 2015 May; 35(5):392-400.

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Abbreviations Used

ATP	adenosine triphosphate
C	cytosine
° C	degrees Celsius
cDNA	complimentary deoxyribonucleic acid
CHC	chronic hepatitis C
CMV	cytomegalovirus
CYP2E1	Cytochrome P450-2e1
DEPC	diethyl pyrocarbonate
dGTP	deoxyguanosine-5'-triphosphate
dH_2O	deionised water
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
dTTP	deoxythymidine-5'-triphosphate
EDTA	ethylene-diamine tetra-acetic acid
ER	endoplasmic reticulum
FACS	fluorescent activated cell sorting
FCS	foetal calf serum
FFA	free fatty acid
FITC	fluorescein isothiocyanate
g	grams
× g	relative centrifugal force (RCF)
GAPDH	glyceraldehyde-3-phosphate deydrogenase
HCC	hepatocellular carcinoma

HCV	hepatitis C virus
HRP	horse radish peroxidise
hr	hour(s)
IRS -1	insulin receptor substrate 1
IRS-2	insulin receptor substrate 2
IFN-α	interferon alpha
IFN-β	Interferon beta
IPS-1	interferon-beta promoter stimulator 1
IFN-γ	interferon gamma
IRES	internal ribosome entry site
ISG	Interferon stimulating gene(s)
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
LIL	liver infiltrating lymphocyte
luc	luciferase
MAVS	mitochondrial antiviral-signalling protein
µg	micrograms
µl	microlitres
µM	micromolar
mA	milliamps

mg	milligrams
ml	millilitres
mM	millimolar
min	minute(s)
mRNA	messenger RNA
MW	molecular weight
ng	nanograms
nM	nanomolar
N/A	not applicable
nt	nucleotide
OA	oleic acid
ORF	open reading frame
PA	palmitic acid
PBS	phosphate buffered saline; 150 mM NaCl, 6 mM K-PO_4, 2 mM KH_2PO_4 (pH 7)
PCR	polymerase chain reaction
PEI	polyethyleneimine
pg	picograms
pmol	picomolar
qRT-PCR	quantitative real-time polymerase chain reaction
RBC	red blood cell
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)
SNP	single nucleotide polymorphism
ss	single stranded
STAT	signal transducer and activator of transcription
SVR	sustained virological response
TAE	0.04 M Tris (pH 8), 0.04 M Acetic Acid, 1 mM EDTA
TEMED	N,N,N',N'-tetramethylethyethylenediamine
TLR	Toll-like receptor
Tris	3,3,5,5-tetramethylbenzidine
TRIF	TIR-domain-containing adapter-inducing interferon-β
TYK2	tyrosine kinase 2
U	units
UTR	untranslated region
V	volts
w/v	weight per volume