Studies on hepatitis C virus replication and pathogenesis

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Introduction

Hepatitis C virus (HCV) infects 170 million individuals and is a major cause of chronic liver disease, including fibrosis, cirrhosis and hepatocellular carcinoma. The virus has a single stranded positive sense RNA genome of 9.5kb that contains a long open reading frame encoding a single polyprotein of 3000 amino acids which is cleaved into 10 individual polypeptides by a combination of host cell and virus specific proteases. We are interested in understanding the molecular mechanisms of viral genome replication and assembly, with a particular focus on the virus-host interactions that underpin these processes. The ultimate goal of this research is to identify new targets for the development of novel antivirals.

Results

A major focus of work is NS5A, a pleiotropic phosphoprotein with multiple roles in the virus lifecycle. We are investigating the role of NS5A in virus replication and assembly, as well as its interactions with cellular factors. For example, recently we have used a mass spectrometric approach to identify sites of phosphorylation within the protein and generated mutants of these phosphorylation sites to characterise the role of this post-translational modification in NS5A function. In collaboration with Grahame Hardie (Dundee) we are raising phosphospecific antibodies to these sites with the intention of probing the role of phosphorylation further. Other studies are investigating the interactions of NS5A with RNA, both *in vitro* and *in vivo*. Similar studies are ongoing with the viral capsid or Core protein with a view to dissecting the mechanism by which new virus particles are assembled.

We also use proteomic and imaging techniques to probe the multiprotein complex that replicates the viral genome (e.g. purifying nascent RNA from infected cells and identifying associated proteins by mass spectrometry, and genetically tagging the virus to enable either high resolution EM or fluorescent imaging). In collaboration with J. Mankouri we are utilising a recently installed confocal microscope with live cell imaging capability located within a category III containment facility – a unique resource within the UK for the study of HCV. This facility is currently being used to understand HCV induced autophagy.

In collaboration with Colin Fishwick (Leeds) we are applying structure-based drug design methodology to the NS2 protein, a key protease involved in the cleavage of the viral polyprotein. We have established a robust cell-based assay to identify small molecules with the ability to block NS2 mediated cleavage. We hope that these may form the basis for a novel future therapeutic approach.

Publications

Mohl, B.-P., Tedbury, P., Griffin, S. & Harris, M. (2012) Hepatitis c virus-induced autophagy is independent of the unfolded protein response. *J. Virol.* **86:** 10724-10732.

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Collaborators

External: G. Hardie (University of Dundee), J. McLauchlan (MRC Virology Unit, Glasgow).
Leeds: J. Mankouri, S. Griffin, C. Peers and C. Fishwick