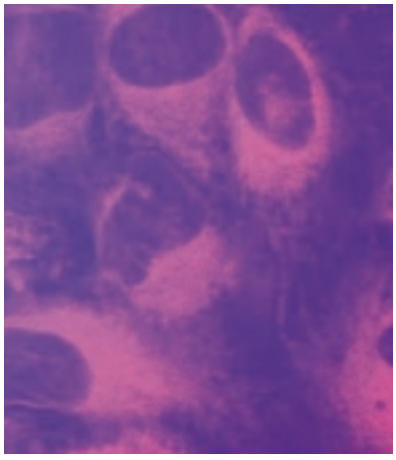

Figure 1. Confocal fluorescence image of severely damaged tumour cells treated with HPPH-doped nanoparticles. Image provided by Paras Prasad of the State University of New York, Buffalo (http://www.photonics.buffalo.edu/).

Prasad also elaborated on an extension of the technique, whereby a magnetic core is encapsulated within a silica nanoparticle and targeted to tumour tissue. The magnetically impregnated tumour cells could then be killed by exposing the tissue to a DC magnetic field. This is a potentially complementary technique to PDT, as Prasad explained, 'If photodynamic drugs can be encapsulated within such nanoparticles, they can work as dual-killers, by the magnetic as well as photodynamic action.'

Promise and potential

So, will PDT finally be associated with words such as 'routine' and 'successful', rather than 'promising' and 'potential'? Stan Brown of the University of Leeds, UK (http://www.leeds.ac.uk/), a PDT expert not connected with the study, has mixed feelings. 'The two fundamental problems to be overcome in developing PDT are to achieve good selectivity and lack of generalized skin photosensitivity,' he said. 'If this approach can achieve either of these then that would represent significant progress.' However, he cautioned that without *in vivo* data, it is not quite clear whether the nanoparticle approach will do everything the authors expect.

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Fgl2: link between hepatitis B and SARS?

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A new clotting factor protein, Fgl2/fibroleukin prothrombinase, has been found to be important in viral infection, including hepatitis B [1], by researchers at the University of Toronto, Canada (http://www.utoronto.ca/).

A greater understanding

Chronic hepatitis B virus affects ~300 million people worldwide [2,3] and a greater understanding of the pathogenesis of viral-induced hepatocellular injury is required. The Fgl2/fibroleukin protein is also triggered by corona virus in mice, which suggests a possible link to the human corona virus that causes SARS.

Almost 80,000 people become infected with hepatitis B virus each year in the USA, despite the fact that it is a vaccine-preventable disease [4]. There are >1 million chronically infected individuals within the USA and these patients are at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. Chronic hepatitis B virus can often lead to severe complications and death after decades of infection.

Fgl2/fibroleukin

Fgl2/fibroleukin is an immune coagulant, which can directly cleave prothrombin into thrombin and has the characteristics of a serine protease. Expression is markedly upregulated by interferon-γ (IFN-γ), which is also important in viral-induced liver disease in humans and model systems. It was initially cloned from CD8+ cytotoxic T cells and shares homology of its carboxyl terminus with fibrinogen β and γ chains.

The viral pathogens that cause liver disease are not all directly cytopathic for the hepatocyte. For example, an immune response to the virus causes the hepatocellular injury that is associated with hepatitis B virus, rather than direct hepatocellular necrosis induced by the virus. Fibrin deposition and thrombosis within the microvasculature of the liver is also important in the immune response to viral infection of the liver. Importantly, the pathways by which vascular

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thromboses and fibrin generation occurs in viral hepatitis might be mechanistically distinct from the classical pathways of coagulation, which are induced by bacterial lipopolysaccharide or mechanical trauma.

Liver disease

The link between Fgl2/fibroleukin and liver disease was identified when a protein with unique clotting ability was isolated from the livers of mice infected with corona virus. That protein was Fgl2/fibroleukin, which, once produced, cleaves prothrombin to thrombin, resulting in a fibrin clot at the site of acute viral infection.

Therefore, this new research has generated Fgl2/fibroleukin-deficient mice to further study and define the role of this protein in the initiation and localization of fibrin deposition in viral hepatitis [1]. The effect of Fgl2/fibroleukin genotype on *in vitro* and *in vivo* responses to murine hepatitis virus type-3 (MHV3) was then assessed, together with Fgl2/fibroleukin expression in patients.

A model of MHV3 – a member of the *Coronaviridae* group of positive-stranded enveloped RNA viruses – was employed and infected mice developed markedly reduced liver necrosis and showed increased survival. Fibrin deposition was also reduced (Figure 1). Thus, hepatocellular injury induced by MHV3 is dependent on the Fgl2/fibroleukin prothrombinase.

The study also shows that Fgl2/fibroleukin mRNA expression and protein production varies markedly in patients with chronic viral hepatitis B versus those with minimal chronic viral hepatitis B, being highly correlated with fibrin expression. Philip Marsden, lead author of the study and Professor of Medicine at the University of Toronto, says, 'Fibrin deposition in tissue is very important and Fgl2/fibroleukin is only now recognised as being important in liver disease'.

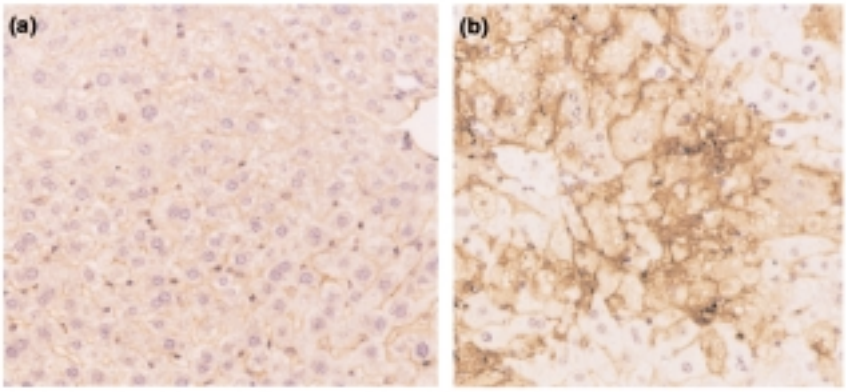

Figure 1. Expression of fibrin in the liver of coronavirus-infected mice. As indicated by the brown staining, fibrin expression is minimal in Fgl2/fibroleukin-deficient mice **(a)** compared to wild-type susceptible mice **(b)**. Fibrin expression correlates with hepatocellular injury. Figure courtesy of Philip Marsden, University of Toronto (http://www.utoronto.ca/).

The promise of drug therapy?

Marsden believes this work paves the way for future therapies and provides a new approach to combating viral disease. 'I am excited by this work and believe it will make a real difference to hepatitis patients', says Marsden. The pharmacological blockade of Fgl2/fibroleukin could offer an important new treatment approach in hepatitis B virus-induced disease. Marsden comments, 'Antibodies are now being generated to neutralize Fgl2 activity, which we hope will be useful in treating patients with viral hepatitis'.

Thomas Lane, Associate Professor, Department of Molecular Biology & Biochemistry, University of California (http://www.ucla.edu/), commented: 'These results are novel and exciting in that they use a genetic approach to clearly show that Fgl2/fibroleukin expression is important in contributing to the pathology of experimental viral-induced hepatitis. While the evidence is compelling, additional work is necessary to determine if this is a safe and effective target. However, these observations lend significant support to arguments that targeting Fgl2/fibroleukin offers a novel method for intervention and treatment for patients with viral hepatitis.'

Applicability to SARS?

With the recent worldwide outbreak of SARS, the relevance of this work in chronic hepatitis B virus infection is timely. However, as Marsden is keen to stress, there is as yet no direct evidence to link Fgl2/fibroleukin and SARS. 'We are now examining patients with SARS for Fgl2 activity and the possible use of neutralizing antibodies for their treatment,' he says. As a logical target for molecular manipulation, the clotting protein offers hope for the development of newer treatment protocols for hepatitis patients. This research opens the way for initial thoughts of a hepatitis treatment by blocking a clotting protein and, if such inhibitors are generated that work in hepatitis patients, perhaps the same inhibitors have the potential to be used in the treatment of SARS.

'[The researchers] found that the MHV3 coronavirus nucleocapsid protein is critical in the transcriptional activation of the gene,' said K.Y. Yuen, a Professor in the Department of Microbiology, Queen Mary Hospital, University of Hong Kong (http://www.hku.hk/). 'At this stage we still have no idea whether the SARS coronavirus infection in human can induce Fgl2/fibroleukin prothrombinase expression. However,

postmortem examination in some HK patients dying from SARS revealed the presence of vascular thrombosis in the pulmonary vessels [5]. Taken together, these findings warrant further investigation of the role of Fgl2/fibroleukin prothrombinase in the pathogenesis of SARS.'

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Fishing for novel drugs

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Marine cyanobacteria are unusual in that they generate molecules in which sub-units of fatty acids are structurally linked to amino acids. This polyketide–peptide backbone generates enormous chemical diversity, which researchers at Oregon State University in Corvallis (OSU; http://oreganstate.edu) are now beginning to mine for novel drug action [1].

Tailored to fit

'There are just two microbial groups that really integrate those two types of pathways with the abundance and frequency that we see in cyanobacteria: the cyanobacteria and the myxobacteria,' said Bill Gerwick, whose group at the College of Pharmacy at OSU, is now screening the products of cyanobacterial secondary metabolism for anti-cancer effects, among others.

The team is also exploring novel 'tailoring' functions by which the cyanobacteria appear to modify the basic polyketide–peptide backbone of these metabolites. 'After the backbone is made, these [tailoring functions] come in and create unusual little functional groups along it,' said Gerwick.

'In particular, we find abundant use of halogen atoms in cyanobacteria. So they incorporate chlorine, bromine and even iodine into some of these molecular structures, and they do so creating chemical functional groups that have really never been seen before in nature.'

Novel therapeutic actions

Not surprisingly, it turns out that those novel functional groups have novel therapeutic actions. Having fractionated a crude organic extract from one Puerto Rican collection of the cyanobacterium *Lyngbya majuscula*, for instance, Gerwick's group succeeded in isolating three new secondary metabolites, including a tryptophan derivative, as well as several potent neurotoxins.

Tryptophan is an essential amino acid that is used in the treatment of migraine, among other things. The neurotoxins could turn out to be useful as tool compounds, says Gerwick, because they could act as biochemical inhibitors in the study of cell biology.

Using MS and NMR spectroscopy to elucidate the structures of the three metabolites, Gerwick's team found that they all showed evidence of this convergent fatty acid-amino acid pathway.

Anti-cancer drugs

Gerwick's lab is currently involved in a large anti-cancer drug-screening program in collaboration with Novartis (http://www.novartis.com) and the National Cancer Institute (http://www.nci.nih.gov), part of the US National Institutes of Health. Three of their compounds have already progressed through this program to clinical trials.

Although their best success to date has been with compounds isolated from sponges, says Gerwick, new research suggests that some of these might actually have been produced by cyanobacteria living symbiotically with the sponges. His team is also following up cyanobacterial candidates for neuroprotective compounds, as well as insecticides, antibiotics and anti-inflammatories.

Some intriguing structures

Burkhard Haefner [2] of the Department of Inflammatory Disease at Johnson & Johnson Pharmaceutical Research and

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