VIROLOGY

Home-grown HCV

Three teams of researchers have developed cell culture systems for the modeling of hepatitis C viral infection and the subsequent generation of infectious virus particles—an important breakthrough in the study of this disease.

Present estimates suggest that 170 million people worldwide are infected with the hepatitis C virus (HCV), a single-stranded RNA virus typically associated with chronic infection of patients, and elevated risk of cirrhosis and hepatocellular carcinoma. HCV has proven difficult to study, owing in part to its narrow host range—only humans and chimpanzees are known to be infected—and the lack of cell culture systems that accurately model the full viral life cycle.

According to Takaji Wakita of the Tokyo Metropolitan Institute for Neuroscience, most existing models rely on strains isolated from chronic carriers. "Only a few HCV strains can replicate in this system," explains Wakita, "and these strains need adaptive mutations in their viral genome to replicate efficiently in cultured cells. We thus thought that it was necessary to isolate an HCV strain from patients other than chronic carriers, because such strains may possess higher replicative capacity." Wakita's breakthrough came when he found a patient with fulminant HCV—a rare and overwhelming pattern of infection that is often fatal. Using samples from this patient, his group isolated an HCV clone, JFH1, subgenomic clones of which proved capable of highly efficient replication in a variety of cell lines.

Using JFH1, Wakita's team has now made another significant breakthrough in HCV research. Working with the groups of Ralf Bartenschlager of the University of Heidelberg and T. Jake Liang at the US National Institutes of Health, they showed that Huh7 human hepatoma cells transfected with full-length JFH1 were capable of producing HCV particles, could then infect new Huh7 cells (Wakita *et al.*, 2005). The viral particles were physically similar to naturally produced virus, and the infection of new cells could be blocked with antibodies against CD81, a putative receptor for viral binding and entry. Notably, the culture-derived virus was also used to infect a chimpanzee, although viral RNA was no longer detectable *in vivo* beyond five weeks after infection.

Wakita's and Bartenschlager's groups presented their initial findings at a conference in Heidelberg, where they caught the attention of members of the lab of Frank Chisari, of The Scripps Research Institute. When they returned and related Wakita's findings, says Chisari, "a light went on inside my head that said, this may be inefficient, but this is really important—this is a very, very important advance." Chisari immediately phoned Wakita, who agreed to collaborate on further studies and sent him JFH1 genomic samples. Chisari's group developed a new cell line, derived from Huh7.5, a Huh7-based cell line originating in the lab of Charles Rice, at Rockefeller University. This cell line proved more suitable for work with JFH1, and after transfection, the cells produced viral RNA and protein much more rapidly than the Huh7 cells (**Fig. 1**), and at sustained levels (Zhong *et al.*, 2005). This system also produced infectious viral particles at higher titers and which were suitable for multiple rounds of passaging without loss of infectivity.

In the meantime, Rice's group was conducting similar work. Rather than working with full-length JFH1, however, they generated chimeric genomes combining JFH1's nonstructural genes with structural gene sequences derived from other strains (Lindenbach *et al.*, 2005). JFH1 is classified as a genotype 2a virus, and when paired with genetic material from J6, another 2a virus, the chimera retained infectivity in Huh7.5 cells—even though full-length J6 is not infectious in culture. By contrast, sequence derived from other genotype classes precluded infection. Chisari hails this as an important finding: "It's a demonstration that the structural proteins of another molecular clone can substitute for the structural proteins of JFH1 and still result in the production of an infectious virus." By swapping genomic components between virulent and nonvirulent strains, it will become possible to identify genetic determinants of viral pathology. "That will be the next frontier," says Chisari, "the next major step in this experimental system."

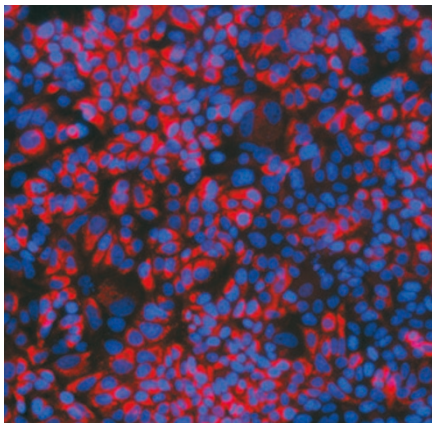

Figure 1 | Huh7.5.1 cells, five days after infection with JH1-derived HCV. Cells have been immunostained for NS5A, an HCV protein (red). Image courtesy of Jin Zhong and Frank Chisari.

Together, these three articles provide strong support for this culture-based approach as a platform for a new generation of investigations into HCV pathology. "This system provides many opportunities to study HCV life cycles, such as virus entry, replication, virus particle formation and virus secretion steps," says Wakita. "Thus, every step will be a target for antiviral drug development."

Michael Eisenstein

RESEARCH PAPERS

Wakita, T. *et al.* Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat. Med.* **11**, 791–796 (2005).

Zhong, J. *et al.* Robust hepatitis C virus infection *in vitro*. *Proc. Natl. Acad. Sci. USA* **102**, 9294–9299 (2005).

Lindenbach, B.D. *et al.* Complete replication of hepatitis C virus in cell culture. *Science*; published online 9 June, 2005.