Stem cell-derived HCV infection systems illustrate the bright future of human hepatocyte research

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Most cells in the body can produce the cytokine interferon (IFN) in response to viral infections. IFN produced by virus-infected cell signals in both autocrine and paracrine fashion through the janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway to activate interferon stimulated genes (ISG). There are over 400 ISG with numerous antiviral functions. Depending on the virus and the cell type, subsets of ISG are induced that help clear the infection. Thus, the IFN-ISG pathways are complex networks that form the first response to viral infections.[1]

For many decades, chronic hepatitis C virus (HCV) infection was the leading cause of advanced liver disease in many nations around the world. Exposure to HCV leads to spontaneous clearance in 20%–30% of individuals, while the majority develop chronic infection that can lead to advanced liver disease. On exposure to HCV, human livers respond by producing a number of ISG. Based on several observations, ISGs have long been postulated to play a key role in HCV clearance. Since the early 1990s high doses of recombinant IFNα became the first effective treatment for HCV infection. For over two decades, various formulations of IFNα remained the backbone of HCV treatment, in spite of being much more effective in acute than chronic infection and with starkly different outcomes depending on the viral genotype. Next a polymorphism in a gene encoding IFNλ4 was strongly associated with spontaneous clearance and to IFNα treatment responses.[2] Since the approval of direct acting antivirals, which can cure the overwhelming majority of chronic HCV patients, baseline ISG expression may still play a role in treatment response in difficult-to-treat populations.[3] These combined observations imply that IFN-ISG pathways play an important role in HCV control.

In Gut, Carpentier *et al*[4] characterised which ISGs are induced by HCV infection in cell culture. Studying HCV in tissue culture has long been plagued by a paucity of systems with intact IFN signalling. Although primary human hepatocytes (PHH) remain the gold standard, these are generally challenging to maintain functional in culture beyond days. Carpentier *et al*, therefore, used hepatocyte-like cells (HLCs) that can reproducibly be differentiated from

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renewable stem cell sources and maintained in culture for weeks. HLCs are less mature than PHH,[5] but they are nevertheless susceptible to most hepatitis viral infections and can mount robust ISG responses. In this study, Carpentier established an HCV infection model, which rapidly induced ISG expression and viral clearance. By contrast, HCV infection in the presence of a chemical inhibitor of the Jak/STAT signalling pathway allowed the authors to establish a chronic infection for weeks. Interestingly, when the inhibitor was withdrawn the ISG response returned and was able to clear the HCV infection. Their detailed characterisation of the ISG in these systems advances insights to which ISG may play a role in hepatocyte clearance of HCV.

It is of interest that Carpentier used an unusual HCV strain (p100) that can achieve high-level viral replication and allegedly partial IFN resistance. This strain was generated from long-term passage of the Jc1 virus (a common laboratory strain) in cell culture and contains multiple mutations in several regions of the vial genome. The molecular basis of the phenotype of this strain has not been adequately elucidated in this study. Thus, it is not clear whether the findings are directly comparable to natural HCV infection.

The HLC models established by Carpentier and by a number of other groups have great potential to advance research into human hepatocyte biology. They carry several striking advantages over PHH, which are generally isolated from discarded liver tissues. First, HLCs are differentiated from stem cells that can be propagated like cell lines, making their supply unlimited. This stands in stark contrast to high-quality PHH, which are scarce and non-renewable. Second, HLC can be generated from individuals with liver conditions. Using technologies to create induced pluripotent stem cells from non-liver cells, HLC can be created from most individuals even when they have advanced liver disease. This again is very different from PHH. Whereas high-quality PHH can be isolated from a subset of healthy livers, diseased livers generally produce poor-quality PHH that cannot be cultured or expanded in liver chimeric mouse models.[6] Third, the revolution in genomic engineering allows for the efficient editing of genes in cell lines. More recently, efficient ways to correct single nucleotide polymorphisms have been developed.[7] These genomic engineering technologies are now widely applied to alter stem cell lines, which can then be selected for expansion and subsequent HLC differentiation. This advance offers the potential to engineer and study HLC with, for example, the IFNλ4 polymorphism associated with spontaneous HCV clearance. Even though PHH freshly isolated from liver chimeric mice can form long-term cultures,[8] such approaches are currently not feasible with PHH as they do not proliferate in cell culture and editing efficiencies remain quite inefficient.

Thus, the work by Carpentier and other groups has opened doors to start investigating the roles of individual ISG in controlling HCV infection. These techniques can now easily be applied to other hepatotropic infections and to numerous other liver diseases. Furthermore, when HLC can reliably and reproducibly expand in liver chimeric mouse models[9] and further mature,[10] these systems will overcome many of the hurdles that have long impeded human hepatocyte research.

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