Hepatology Snapshot

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Chimeric mouse model of hepatitis B virus infection

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Introduction

Chronic infection with hepatitis B virus (HBV) continues to be a major health problem affecting about 400 million people worldwide. The narrow species tropism of HBV has hindered progresses in HBV research and development of more efficient treatments. Due to strong restraints encountered using chimpanzees and animal models based on HBV-related viruses, recent developments focused on using the natural target of HBV infection: the human hepatocyte. However, *in vitro* susceptibility of human hepatocytes to HBV is limited and cultured cells may respond differently to the infection and other stimuli than hepatocytes in the liver. Attempts to create mice harboring human chimeric livers led to the establishment of two major models: the urokinase-type plasminogen activator (uPA) transgenic mice [8] and the knockout fumarylacetoacetate hydrolase (Fah) mice [1]. Common features of these systems are: (i) a transgene-induced hepatocyte damage creating space and regenerative stimulus for the transplanted cells to repopulate the host livers, and (ii) the absence of adaptive immune responses permitting xenogenic transplantation. Both models are amenable of infection with HBV [2,3]. Here we present recent advances and applications of the uPA model, the most used and best characterized chimeric model currently available for HBV infection studies and preclinical drug evaluation.

Generation of human chimeric uPA/SCID mice

(1) Expression of a liver-toxic uPA transgene, driven by an albumin promoter, induces subacute liver failure in newborn animals, promoting strong growth advantage for transplanted hepatocytes [8]. Although transgene deletion takes place in individual mouse hepatocytes, inactivation of both transgene alleles in the same cell is a rare event. Consequently, mortality of homozygous uPA mice is high, unless mouse livers are reconstituted with healthy hepatocytes. As shown in Fig. 1, mating uPA mice with mouse strains lacking functional B- and T-cells, like Severe Combined Immune Deficient (SCID) mice, permits human hepatocytes engraftment [3,7].

(2) One million human hepatocytes isolated from surgical liver remnants or explanted livers are injected intra-splenically into 2- to 3-week-old homozygous uPA/SCID mice. The use of high-viable cryopreserved-thawed hepatocytes [5,10] permits to generate a large number of mice displaying high levels of human chimerism on demand, thus overcoming previous limitations due to the narrow time frame available for transplantation.

(3) One week post-transplantation small clusters of human hepatocytes start to proliferate within the pale mouse liver, as shown by human specific cytokeratin-18 and Ki-67 double staining, and expand to constitute larger regenerative nodules which eventually merge together, replacing the diseased liver parenchyma in variable proportions. Mouse liver reconstitution takes 8–10 weeks and can be monitored by measuring human albumin concentrations in mouse serum [5].

Current and potential applications of the model for HBV studies

(4) While mouse hepatocytes do not support HBV infection, human-chimeric mice can be efficiently infected by injecting infectious serum derived from either patients or chimeric mice. Furthermore, genetically engineered viruses created in cell culture can be used to investigate phenotype and *in vivo* fitness of distinct HBV genotypes and variants [9].

(5) HBV spreading phase: Establishment of HBV infection requires the formation of the cccDNA minichromosome, the HBV transcriptional template. This step is achieved, initially, in very few human hepatocytes. Three weeks post-infection only sporadic cells stain HBcAg-positive, while at week 6 the majority of human hepatocytes are infected. Thus, several weeks are needed for HBV to spread among human hepatocytes, as documented by the gradual increase of HBcAg-positive human hepatocytes. Within this time frame the system offers unique possibilities to study the early phase of infection, as well as for preclinical evaluation of agents interfering with viral entry, such as HBV-derived peptides [7] and neutralizing antibodies, or with cccDNA formation.

(6) HBV chronic phase: When HBV spreading is accomplished, nearly all human hepatocytes stain HBcAg-positive and viremia

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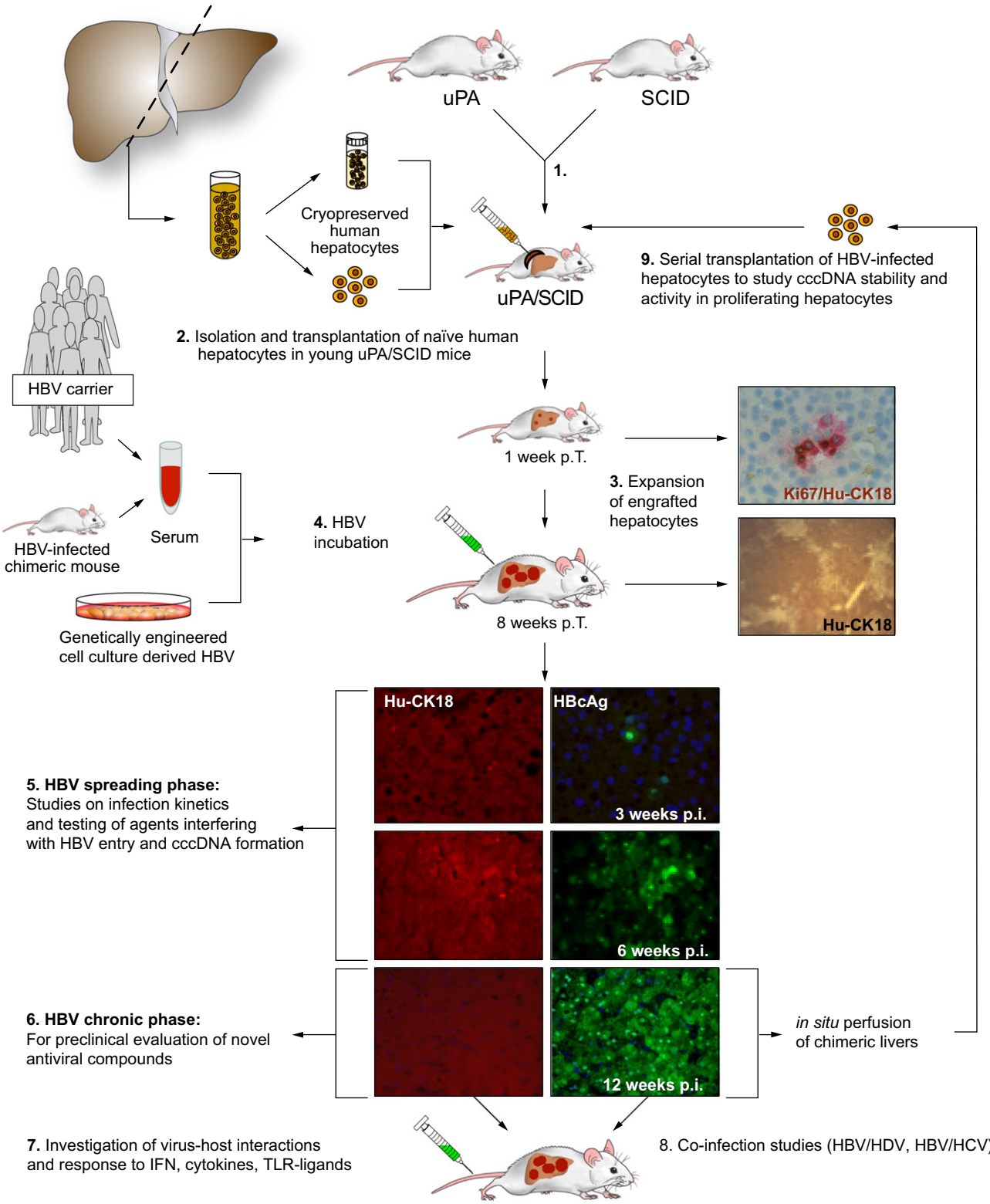

Fig. 1. Current and potential applications of human liver chimeric uPA/SCID mice for HBV infection studies and preclinical antiviral drug evaluation.

reaches a stable plateau ranging from 10^{-7} to 10^{-9} HBV-DNA copies/ml, which fairly correlates with the levels of human chimerism. Mice with 10–70% repopulation levels display stable HBV titres (up to $5x10^{-8}$) and thus are ideal to perform long-term antiviral testing, while higher human repopulation levels lead to renal insufficiency and lower mouse survival rates.

(7) Within the mouse liver human hepatocytes maintain a functional innate immune system and respond to stimuli induced by exogenously applied human IFN-α. The lack of an adaptive immune system and the undetectable responsiveness of mouse liver cells to human IFN-α make the model ideal to exploit the capacities of HBV to interfere with pathways of the innate antiviral response in human hepatocytes [5].

(8) Chimeric mice can be super-infected or simultaneously infected with different human hepatotropic viruses to investigate mechanisms of virus interference and response to antiviral treatment in the setting of co-infection [4].

(9) Serial passage of human hepatocytes in uPA/SCID mice may enable investigation of the impact of hepatocyte proliferation on cccDNA stability and activity *in vivo* [6].

The feasibility of generating uPA/SCID mice containing both human liver and immune cells awaits investigation.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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