Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Review

Seeds in the liver

Hongjie Ji[a,b], Yanrong Lu[b], Yujun Shi[a,b,*]

[a] Laboratory of Pathology, West China Hospital, Sichuan University, Chengdu, China
[b] Key Laboratory of Transplant Engineering and Immunology, NHFPC, West China Hospital, Sichuan University, China

ARTICLE INFO

Keywords:
Lineage tracing
Cre/LoxP
Hepatic progenitor cell
Seed cells

ABSTRACT

The liver is a crucial organ for homeostasis and has a tremendous self-renewal and regenerative capacity. It has long been believed that the self-renewal and repair of the liver within a given physiological condition or its repopulation in chronic liver diseases, when hepatocyte proliferation is impaired, will primarily be conducted by the proliferating duct cells, termed "oval cells" or hepatic progenitor cells (HPCs). In addition, numerous studies have revealed that HPCs are the initial tumor cells of liver cancer under certain micro-environments. However, benefit from the extensive application of lineage tracing strategies using the Cre/LoxP system, researchers have redefined the fate of these bipotential cells, raising obvious controversies regarding the capacity of liver cells to control their own biology and differentiation. Here, we review the relevant articles, focusing on cell-lineage tracing to better understanding seed cells and their distinct fate in the liver.

1. Background

The liver is one of the body's most multifunctional organs, controlling glycolytic and urea metabolism, cholesterol levels, blood detoxification, and the biosynthesis of pivotal hormones and proteins. The most intriguing and mysterious character of the liver is its ability to quickly self-regenerate or repair in response to acute liver mass loss or chemical-induced injury, an ability that has been comprehensively confirmed in animal experiments using partial hepatectomy (PHx) or chemical injection in rodents (Fausto et al., 2006; Fausto et al., 2012; Taub, 2004). This feature is utilized in clinical scenarios in which PHx is carried out to resect liver tumors. In the case of living-donor liver transplantation, in which a portion of the liver is taken from a healthy donor and transplanted into a recipient, both remnants will regrow into a functional liver mass. This process is accomplished via division of the hepatocytes and cholangiocytes within the remnant liver. These cells leave their normal mitotically quiescent state, termed the "G0" phase, and enter the cell cycle in a semi-synchronized fashion. Thus, in these situations, mature hepatocytes are the true seed cells in the renewal of the liver's architecture or biological function.

However, the regenerative capacity of the mature hepatocytes is continually and severely compromised during the development of diverse chronic liver diseases, such as non-alcoholic fatty liver disease (Carpino et al., 2013; Yang et al., 2004) and chronic viral hepatitis (Marshall et al., 2005). In this scenario, hepatic progenitor cells (HPCs) become activated and spontaneously copy themselves. The status and expansion pattern of HPCs have been reviewed elsewhere (Duncan et al., 2009; Miyajima et al., 2014). During the past 20 years, it has long been believed that HPCs are seed cells in the liver that have the ability to regenerate biliary and hepatocellular epithelium in chronic liver diseases in vivo (Español-Suñer et al., 2012; Fellous et al., 2009; Wang et al., 2003), a belief strengthened by in vitro clonogenicity and multilineage differentiation. Furthermore, HPCs have been generally considered as origin/founder cells of liver cancer stem cells or hepatocellular carcinomas (HCCs) (Dorrell et al., 2011; Huch et al., 2013; Okabe et al., 2009). The possible cell source of hepatocytes and HCCs is illustrated in Fig. 1.

HPCs, as putative adult liver stem cells, are often thought to reside within the canals of Hering, the terminal branches of the biliary tree, and are quiescent in healthy liver (Paku et al., 2001). Due to the lack of a powerful lineage-specific tracing system in the adult liver, much of our understanding regarding the biology and differentiation of HPCs

Abbreviations: ANIT, alpha-naphthyl isothiocyanate; APAP, acetaminophen; ASC, activated stellate cells; ASH, alcoholic steatohepatitis; BDL, Bile duct ligation; BW, body weight; CC, cholangiocytes; CCl_4, carbon tetrachloride; CDE, choline-deficient ethionine-supplemented; CK19, Cytokeratin 19; CMD, Choline-methionine deficient; CPS, carbamoyl-phosphate synthetase 1; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DEN, Diethylnitrosamine; ER, estrogen receptor; FLPC, fetal liver progenitor cells; GS, glutamine synthetase; HB, hepatoblasts; HCC, Hepatocellular carcinoma; HC, hepatocytes; HFD, high fat diet; HNF1β, hepatic nuclear factor 1β; HNF4, αHepatic nuclear factor 4 alpha; HPCs, hepatic progenitor cells; HSC, hepatic stellate cells; Hsp90, Heat shock protein 90; iDTR, induced diphtheria toxin receptor; IRC, interferon responsive cells; MUP-uPA, major urinary protein-urokinase plasminogen activator; NASH, non-alcoholic steatohepatitis; OPN, osteopontin; PFB, portal fibroblasts; PHx, partial hepatectomy; PMC, periportal mesenchymal cells; R-H, resistant-hepatocyte; SOX9, Sry HMG Box Protein 9; STAM, stelic animal model; VSMC, vascular smooth muscle cells

* Corresponding author at: Laboratory of Pathology, West China Hospital, Sichuan University, 37 Guoxue Road, Chengdu 610041, China.
E-mail address: shiyujun@scu.edu.cn (Y. Shi).

http://dx.doi.org/10.1016/j.acthis.2017.03.006
Received 23 January 2017; Received in revised form 28 February 2017; Accepted 21 March 2017

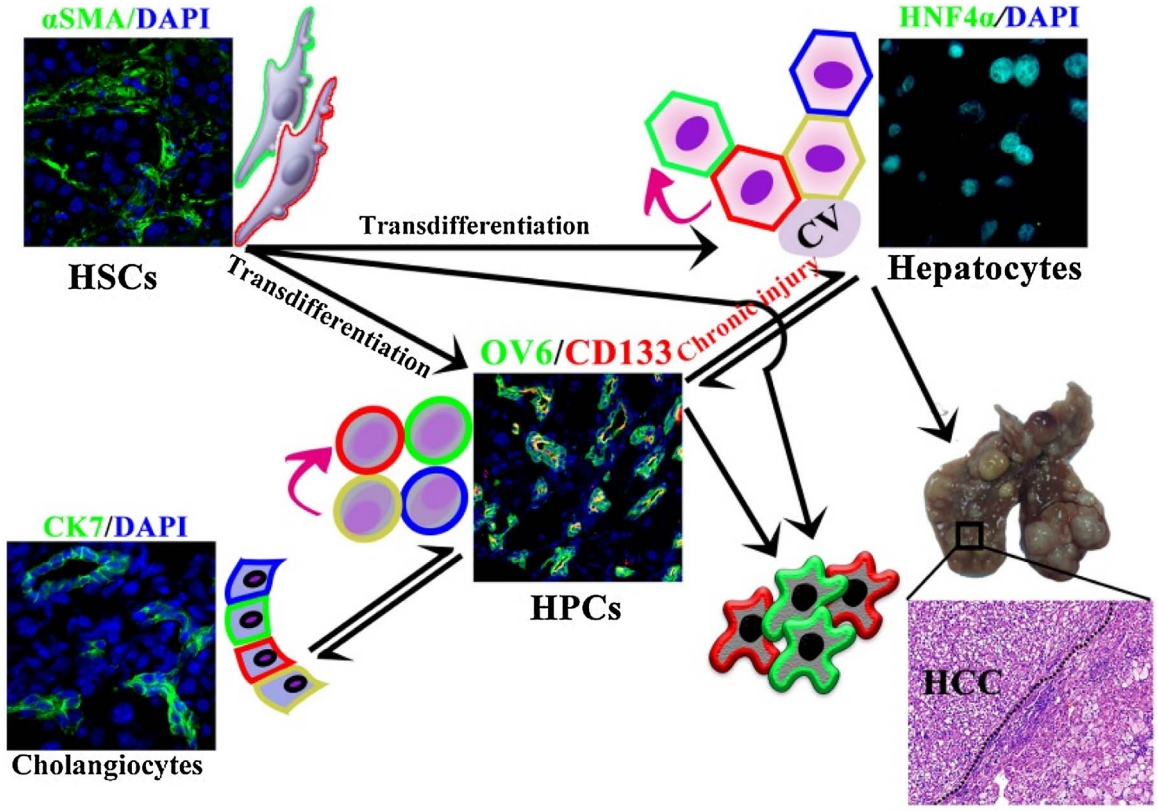

Fig. 1. The possible cell sources of hepatocytes and HCC.

has largely been defined in vitro. HPCs are recognized and isolated by a series of HPC-specific markers, including Sry HMG Box Protein 9 (SOX9), osteopontin (OPN), Cytokeratin 19 (CK19), hepatic nuclear factor 1β (HNF1β), etc. In the past five years, by taking advantage of the genetic modulation strategy and the utilization of the Cre/LoxP system, several mysteries of HPCs have gradually been unfolded.

2. Cell-tracing strategies in the liver

The contributions of different cell types to tissue turnover and regeneration are difficult to address in adult organs. Before the use of the Cre-dependent animal model, the proliferating cells and their progeny in the liver were mainly labeled by tritiated thymidine or BrdU (Evarts et al., 1996; Evarts et al., 1987). The lack of distant progeny labeling ability and cell-type specificity, together with the potential impact of long-term exposure to radio-labeled tracers on cell fate, greatly limited the use of these strategies.

The Cre-dependent system makes it feasible for marking of a specific cell type and its progeny (Greenhalgh et al., 2015; Lemaigre, 2015). In this strategy, one traced cell harbors two separate transgenic components. The first element is Cre recombinase expression under the control of a cell-specific regulator or promoter, in which the Cre gene is fused with a mutated ligand-binding domain of the estrogen receptor (ER) sensitive to tamoxifen. Upon the presence of tamoxifen, CreER becomes disassociated with Hsp90 (Heat shock protein 90) and is translocated from the cytoplasm to the nucleus; upon translocation, a fragment of the flanked stop cassette is eliminated, inducing expression of the second transgene. The latter component codes a reporter protein, such as EGFP, YFP, Tomato, ZsGreen, or Laz, which is controlled by the ROSA26 locus after deletion of the stop cassette. Upon the administration of tamoxifen, these cells and their progeny are marked by reporter proteins, which is the basic strategy of permanent lineage tracing in the liver, as demonstrated in Fig. 2, although non-inducible Cre is used in some cases. Table 1 lists the Cre-dependent lineage tracing system used in this area.

3. HPCs do not spawn hepatocytes efficiently during the chronic injury process

Numerous studies have confirmed that biliary-derived conventional HPCs can form cell clones and differentiate into functional hepatocytes and cholangiocytes *in vitro*, however, it is still in debate that whether HPCs are involved in regeneration and give rise to hepatocytes in chronic liver diseases *in vivo*. It was infeasible to specifically trace HPCs and their progeny *in vivo* until Linda Greenbaum and colleagues began to trace the Foxl1 positive HPCs which are activated immediately after adult liver injury using a Cre system (Sackett et al., 2009). Using the Foxl1-Cre & ROSA26–LacZ or Foxl1-Cre & ROSA26–YFP tracing system, they found that these foxl^{+} lineage cells gave rise to a very small portion of the hepatocytes (approximately 0.5%) when the mice were fed with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), which induces chronic liver injury and initiates ductular reaction (Shin et al., 2015).

Similarly, Tarlow et al. found that SRY-related HMG box transcription factor 9 positive (Sox9 +) ductal progenitor cells contributed only minimally (< 1%) to the hepatocyte pool in DDC-, CDE-, and CCl_4-induced chronic liver injury models, despite these cells have a strong capability of organiod formation in vitro (Tarlow et al., 2014a), which was separately proved by studies from Simone Jörs and Yanger's groups (Jörs et al., 2015; Yanger et al., 2014). In their studies, HPCs labeled by HNF1β and CK19, two putative HPC markers, remained quiescent in homeostatic livers and only contributed to clonal oval cell proliferation in ductular reactions. Although the lineage strategy of these HPCs also showed little "leakage" (labeling cells without tamoxifen injection), the percentage of these HPC-derived hepatocytes was no more than 2%, even in the CDE injury model (Español-Suñer et al., 2012; Rodrigo-Torres et al., 2014).

Moreover, results involving mature hepatocytes rather than HPCs