Editorial

Stem Cell INVESTIGATION

Which is better source for functional hepatocytes?

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The liver performed many physiological functions essential for life. Despite of its strong regenerative capability, the liver cannot fully restore its physiological functions when it is exposed to chronic liver injuries. Since most of liver functions depend on hepatocytes, it is crucial to compensate the lost functions by supplying functional cells for treatment of liver diseases (*Figure 1*). Liver transplantation is an effective therapy for patients suffering from severe liver diseases, though the number of donor is absolutely limited. Alternatively, cell transplantation has been considered as a therapeutic treatment. Therefore, researchers have been working to find a good source for generating hepatocytes *ex vivo* and establish protocols inducing hepatic functions comparable to mature hepatocytes (MHs).

It has been generally considered that in severely damaged liver, liver stem/progenitors (LPCs) or oval cells are activated and supply new hepatocytes for regeneration of liver tissue. Actually, LPCs have been prospectively isolated as $CD133^+$, $EpCAM^+$, or $Thy1^+$ cells and shown to differentiate into hepatocytes and biliary epithelial cells (BECs) *in vitro* and *in vivo* (1). However, it is still under debate whether those LPCs efficiently differentiate into MHs and majorly contribute to liver regeneration.

In addition to LPCs, recent works demonstrate that MHs have a potential to behave hepatocyte progenitors; in chronically injured livers, MHs or part of MHs de-differentiated or converted to progenitor cells that possessed strong capability of proliferation and re-differentiation (2-4). These results show a possibility that MHs or their subfraction could be cellular sources for generating functional hepatocytes *ex vivo*. Indeed, Katsuda *et al*. reported a new approach to generate functional hepatocytes starting from MHs *ex vivo* (5). As generally recognized, MHs cannot continuously proliferate *in vitro*. To overcome this obstacle, they searched an optimal culture condition in which MHs continuously proliferate. Based on previous findings that small compounds contribute to inducing and maintaining various types of stem cells (6), they found that rat MHs acquired strong proliferative capability in the presence of Y-27632 (Rock inhibitor), A-83-01 (ALK inhibitor), and CHIR99021 (GSK3 inhibitor, potential activator for the Wnt/β-catenin pathway). They termed this combination of compounds as YAC. These chemically induced liver progenitor cells (CLiPs) differentiated to MHs and BECs *in vitro*. CLiPs could be stably cultured on Matrigel with YAC for long-term: they kept proliferative capability and bi-directional differentiation potential. Furthermore, CLiPs repopulated the liver of urokinase-type plasminogen activator (uPA)/severe combined immunodeficiency disease (SCID) mice, in which recipient hepatocytes are continuously damaged by uPA. Although CLiPs exhibited aneuploidy and chromosomal translocation, CLiPs could not proliferate upon withdrawal of YAC, indicating they did not transform to oncogenic cells. While aneuploid hepatocytes exist in the healthy liver, it remains unknown how CLiPs can stably keep proliferative capability and bidirectional

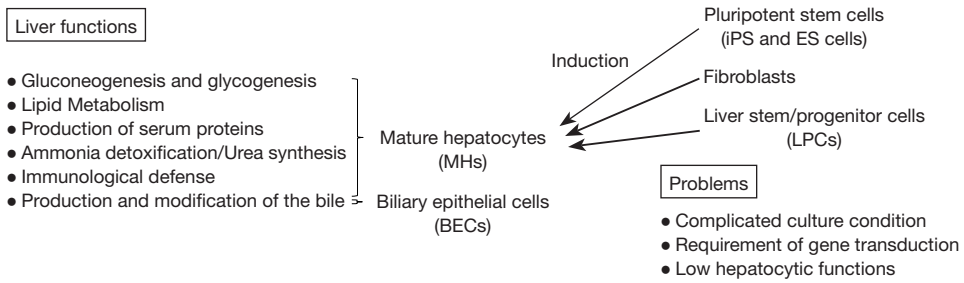

Figure 1 Induction of hepatocytes from stem/progenitor or somatic cells. The liver contains two types of epithelial cells namely mature hepatocytes (MHs) and biliary epithelial cells (BECs). MHs perform most of liver functions. Pluripotent stem cells, liver stem/progenitor cells (LPCs), and fibroblasts are used to generate functional hepatocytes. Complicated culture conditions must be applied for inducing hepatocytic cells from pluripotent stem cells, whereas transcription factors crucial for hepatocyte differentiation are introduced to fibroblasts for the induction. LPCs are highly proliferative but not efficiently differentiate to MHs.

differentiation potential with chromosomal abnormalities including chromosomal translocation. Collectively, their results indicate that MHs are a good cellular source for producing MHs *ex vivo*, though it would be preferable to select CLiPs with the normal karyotype for future applications.

Do MHs uniformly possess plasticity to acquire progenitor characteristics in an appropriate culture condition or in response to certain hepatic injuries *in vivo*? Actually, we reported that a subfraction of rat and human hepatocytes, namely small hepatocytes (SHs), proliferate *in vitro* and re-differentiate to MHs (7,8). We recently enriched mouse hepatocyte progenitors in $CD31^{-}CD45^{-}$ $EpCAM^{-}ICAM\text{-}1^{+}$ fraction (9). $ICAM\text{-}1^{+}$ hepatocytes were mostly mono-nucleated cells, which consisted of diploid and tetraploid ones. Similar to CLiPs, $ICAM\text{-}1^{+}$ hepatocyte progenitors continuously proliferated and efficiently differentiated into MHs *in vitro* and *in vivo*. Katsuda *et al*. first performed live cell imaging and demonstrated that not only mononuclear but also binuclear MHs proliferated in the presence of YAC, suggesting either of them could be the source for CLiPs. However, they further cultured diploid, tetraploid, and octoploid hepatocytes isolated by FACS and concluded that diploid hepatocytes are the major origin of CLiPs. These results suggest that MHs are heterogeneous cell population at least in terms of cellular plasticity, which is correlated with ploidy. However, it remains unknown what determines whether MHs truly terminally differentiated or still possess plasticity regaining progenitor characteristics.

In addition to the induction of CLiPs from rats (rCLiPs), Katsuda *et al*. induced CLiPs from mice (mCLiPs). However, rCLiPs and mCLiPs unlikely possess identical differentiation potential. Consistent with the results of *in vitro* cultures, 2 out of 3 rCLiPs derived from three independent donor rats were engrafted not only as MHs but BECs. The plasticity of rat MHs was previously demonstrated; spheroids consisting of MHs formed biliary tubular structures in collagen gel in the presence of TNFα (10). As compared to rCLiPs, mCLiPs did not efficiently differentiated to BECs *in vitro*. Similar to mCLiPs, mouse $ICAM\text{-}1^{+}$ hepatocyte progenitors showed very limited potential to differentiate to BECs. Even though mice used as the recipients were different, $ICAM\text{-}1^{+}$ hepatocytes were engrafted as MHs but not BECs (*Figure 2*). These results suggest that the progenies of mouse MHs kept identity as hepatocytes even after long-term culture more tightly as compared with rat ones. It may be interesting to investigate what molecular mechanisms determine differentiation potential of rCLiPs and mCLiPs, which may give us an idea how the lineage plasticity of MHs is controlled.

The results provided by Katsuda *et al*. demonstrate that MHs or their subfraction are a good source for generating a mass of functional hepatocytes. Hepatocytes have been also induced from embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) by optimizing culture conditions (11,12) or from fibroblasts by introducing crucial transcription factors (13,14) (*Figure 1*). Although it is impossible to directly compare hepatic functions among those induced hepatocyte-like cells, CLiPs have superior points to pluripotent stem cells and fibroblasts at least in rodents considering the simple culture condition without genetic manipulation. Given that a goal would

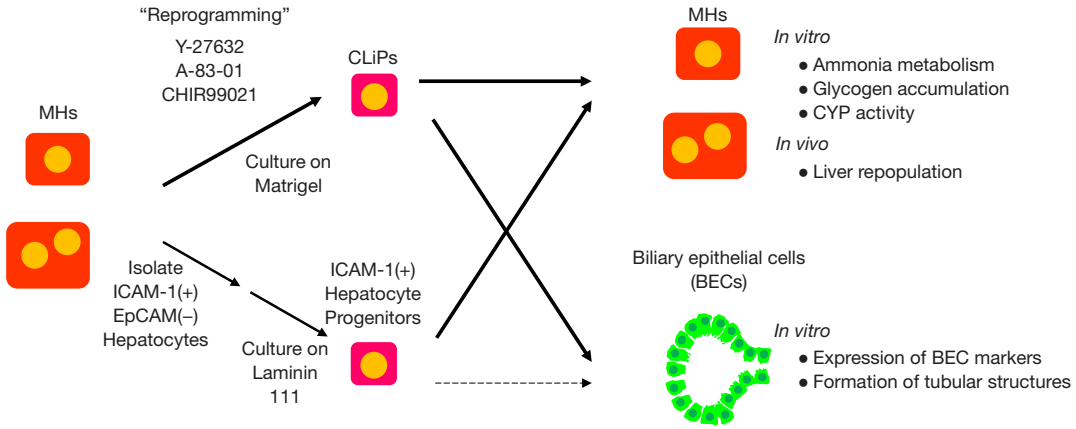

Figure 2 Generation of functional hepatocytes *ex vivo* depending on the plasticity of mature hepatocytes (MHs). MHs or part of MHs acquire proliferative capability and can be expanded *in vitro*. Matrigel or its major component laminin 111 may be important for maintaining proliferative and differentiation potential of chemically induced liver progenitor cells (CLiPs) and ICAM-1$^+$ hepatocyte progenitors during long-term culture. Either from rats or mice, the progenies of MHs differentiate to functional hepatocytes both *in vitro* and *in vivo*. Furthermore, rat cells can also differentiate to biliary epithelial cells (BECs).

be generating human functional hepatocytes *ex vivo*, it is intriguing whether the similar protocol can be applicable to human MHs to generate hCLiPs.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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