

Cell sources for regenerative medicine of the liver and endoderm organs: strategies and perspectives

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In a recent paper, Wang and colleagues showed that a set of defined small molecules plus mesenchymal feeder cells can convert human gastric epithelial cells into induced endodermal progenitors with the capability to differentiate into liver and pancreatic adult parenchymal cells (1). This manuscript could open new perspective in the field of cell therapies for endodermal organs suggesting the stomach as a possible new cell source for the support of liver and pancreatic functions.

In the field of liver diseases, orthotopic liver transplantation (OLT) represents the only curative treatment for acute liver failure and end-stage chronic liver disease (2). However, OLT is limited by severe shortage of organ donors and nearly 15% of patients die on the waiting list (3,4). Therefore, the development of cell therapy strategies represents an interesting curative approach and could represent a possible option to support liver function in the waiting of transplantation.

Cell therapies with mature hepatocytes have provided encouraging results in several clinical trials and adult hepatocyte transplantation has been indicated as a promising alternative to OLT for the treatment of some liver-based metabolic disorders or acute liver failure (5); unfortunately, the effects of hepatocyte transplantation are transient and cell function appears to decline after few months; moreover, cell transplantation procedures cause complications and require immunosuppression (5). Actually, one of the

main obstacles to the clinical application of hepatocyte transplantation resides in sourcing of cells and in difficulties in cryopreservation, and in low engraftment (5). Isolation of primary human hepatocytes competes with organs for OLT; therefore, current sources of tissue for hepatocyte isolation are limited and of marginal quality, such as adult organs that are unsuitable for OLT, livers that have undergone aggressive therapies, or surrounding liver parenchyma obtained after tumor excision (5). As regard, cryopreserved cells, absence of proliferation and reduced functions of thawed hepatocytes dampen their potential for cell therapies (5).

The improvement of regenerative medicine approaches for liver diseases requires the identification of sustainable and readily available cell sources (2). In keeping, the possibility of reprogramming adult somatic cells attracted attention for the possibility to generate mature hepatocyte readily available for transplantation (4). In this field, numerous advances have been obtained since the reprogramming of human fibroblasts into induced pluripotent stem cells (iPSC) using a retroviral vector by Yamanaka and associates (6); up to date, human iPSC lines can be generated not only using fibroblasts cultured from a skin biopsy but additional cell types have been proved to be susceptible to reprogramming such as keratinocytes, blood cells, and mesenchymal stem cells (MSC) (4). In parallel, significant advancements have been obtained in

the definition of protocols for the differentiation of human iPSC into functional mature hepatocytes (iPSC-Hep). Interestingly, all the published protocols recapitulate the major developmental steps leading to liver organogenesis with initial differentiation of iPSCs into definitive endoderm, then hepatic specified endoderm, followed by the formation of hepatic progenitors and finally differentiation into functional hepatocyte-like cells (7).

A step forward has been made by the demonstration that reprogramming strategies do not require the establishment of stable iPSC lines but mature hepatocytes (iMPC-Heps: induced multipotent progenitor cell-derived hepatocytes) can be obtained after partial reprogramming to pluripotency followed by directed hepatocyte differentiation (8). More recently, induced hepatocytes (iHeps) have been directly reprogrammed from fibroblasts (or MSCs) by forcing the expression of hepatic transcription factors (6); Huang *et al.* were able to generate iHeps using three hepatic transcription factors (9); similarly, Du *et al.* generated functional iHeps from fibroblasts using six hepatic transcription factors in combination with MYC overexpression and TP53 knockdown (10).

The clinical application of reprogrammed cells raises concerns about their actual use and the open possibility that these cells could undergo reversion to more primitive state with uncontrolled tumorigenic expansion within the recipient (2). In particular, a critical point for the translation in clinics resides in the frequent occurrence of chromosomal and subchromosomal abnormalities in reprogrammed cells (4). In addition, genomic abnormalities have been reported characterized by the frequent presence of regions with copy number variations of various sizes, either deletions or duplications (4). Moreover, the number of mutations in a human iPSC line is not stable and long term culture may impact the genomic integrity of the cells (2,4). In addition to genomic abnormalities, it is well described that reprogramming is also associated with epigenetic changes (11). These aspects could be particularly crucial for pediatric liver diseases in the light of long life expectancy and in chronic liver diseases since these conditions were characterized by an “adverse niche” characterized by a chronic inflammatory milieu which can favor tumorigenesis (2).

The optimal reprogramming method with no impact on the cell genome is not yet established and reprogrammed cells lack sufficient maturity for widespread clinical use (2,5). In keeping, the small molecule-based reprogramming has attracted much interest due to its

safety and efficiency in controlling cell fates (12); this methodology has been successfully used for fibroblast reprogramming into iPSC or neurons (13). In this context, the recent paper by Wang *et al.* described a novel approach to generate human induced endodermal progenitor cells (hiEndoPCs); the lineage reprogramming of gastrointestinal epithelial cells was obtained using a cocktail of defined small molecules in association with the support of tissue-specific mesenchymal feeders (1). In terms of transcriptional and epigenetic signatures, hiEndoPCs have been proved to be a particular stage of endodermal progenitor cells which is intermediate between primitive gut-tube and posterior foregut (1). *In vitro*, hiEndoPCs clonally expand in culture and have multipotent capability when treated with defined soluble molecules; to this latter regard, hiEndoPCs can give rise to hepatocytes, pancreatic endocrine cells, and intestinal epithelial cells. Interestingly, the authors showed that hiEndoPC-derived hepatic cells are fully functional by testing *in vitro* properties and *in vivo* capability to rescue liver functions in a murine model of tyrosinemia type I liver disease. Importantly, hiEndoPC maintained a normal karyotype during five continuous passages *in vitro* and showed no tumors formation over 6 months after subcutaneous transplantation in mice, thus suggesting minimal tumorigenic potential capacity if compared to iPSC or embryonic stem cells (1). Since human gastric epithelial cells are readily available from human donors of many ages, the conversion strategy proposed by Wang and associates can generate clonally expandable cell populations with a variety of potential applications, including disease modeling and personalized drug screening; moreover, their low tumorigenic potentiality makes them more acceptable for future clinical applications. However, extensive pre-clinical studies in experimental models are needed before reprogrammed cells and their derivatives can be used in human therapy. This is mostly due to the fact that all these cells need several *in vitro* manipulations to generate mature cells; thus, safety issues and the definition of actual protocols should be strictly addressed to meet criteria for translation into clinical programs (2,3).

Besides cell reprogramming, the use of MSCs or determined stem/progenitor cells isolated from adult or fetal human organs has been proposed for the regenerative medicine of the liver (3). Adult or fetal stem cells have the advantage to require only minimal manipulation with respect to reprogrammed cells. In keeping, MSCs are easily sourced, readily cryopreserved, and involve transplantation procedures with minimal, if any, complications (3).

Therefore, several clinical trials based on the use of MSCs in the treatment of liver diseases have been performed (3). The obtained results clearly indicated that the therapy with MSCs in patients affected by liver diseases offers only short-term effects consisting in alleviation of disease symptoms; inefficient lineage restriction to mature hepatocytes has been observed in clinical trials and confirmed in experimental models; actually, the beneficial effects of MSC transplantation have been ascribed to immuno-modulatory properties and paracrine signaling mechanisms (3).

On the other hand, determined stem/progenitor cell population resides into the adult and fetal human liver; in adults, hepatic stem/progenitor cells (HpSCs) are located within the canals of Hering and bile ductules; HpSCs are facultative stem cells characterized by a unique phenotype and able to differentiate into functional mature hepatocytes and cholangiocytes (14). In fetal liver, HpSCs form the ductal plate and give rise to the generation of cholangiocytes of interlobular bile ducts and to periportal hepatocytes during liver development (14). HpSCs can be easily isolated from human livers on the basis of the expression of specific surface antigens such as Epithelial Cell Adhesion Molecule (EpCAM) (15). Moreover, a single Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5)-positive liver stem cell can be expanded to form epithelial spheroids *in vitro* and was able to differentiate into functional hepatocytes *in vitro* and *in vivo* (14,16). Organoids containing Lgr5+ cells were able to be expanded *ex vivo* and to give rise to hepatocytes and cholangiocytes (16). Long-term expansion of adult bile duct-derived bipotent progenitor cells has been obtained from human liver (17,18). A key feature of the expanded cells is the high stability at the chromosome and structural level and the single base changes occur at very low rates (16). Expanded cells can readily be converted into functional hepatocytes *in vitro* and upon transplantation *in vivo* (16). Therefore, these properties indicated HpSCs isolated from adult or fetal human livers as possible source for cell therapy program. However, few clinical trials were started with the use of human HpSCs. To date, the only completed trial is the one under the management of Dr. Chittoor Habibullah and his associates at the Liver Institute in Hyderabad (India) (19). This trial used as cell source human HpSCs and hepatoblasts isolated by EpCAM sorting from fetal livers. These investigators focused on patients affected by liver diseases of different etiologies such as biliary atresia, inborn errors of metabolism (Crigler-Najjar), non-alcoholic steatohepatitis, viral cirrhosis, alcoholic cirrhosis

and drug toxicity. Results obtained by Habibullah and coworkers suggest that human HpSCs can be effective in treating patients with liver disease. A representative early publication concerned a trial of 25 subjects and 25 controls with decompensated liver cirrhosis due to various causes; subjects received fetal liver-derived EpCAM+ cell infusions into the liver via the hepatic artery. At a 6-month follow-up, multiple diagnostic and biochemical parameters showed clear improvement, and there was a significant decrease in the patients' MELD scores (19). In western countries, an analogue clinical trial using cells isolated from fetal livers was started (20). Remarkably, immune suppression was not required, although donors and recipients were not matched for histocompatibility antigens. This could be due to the intrinsic immunomodulatory properties of liver stem cells (21).

Another possible source for stem/progenitor cells for liver regenerative medicine is represented by the extrahepatic biliary tree, including the gallbladder and the hepato-pancreatic ampulla (22,23). A key advantage is their ready availability from adult or fetal donors and from cholecystectomized patients in comparison with the scarcity of marginal livers usable for the isolation of adult HpSCs. Extrahepatic biliary tree contains a large population of progenitor/stem cells named biliary tree stem/progenitor cells (BTSCs) (17). From an anatomical point of view, BTSCs reside within peribiliary glands (17); embryologically, they represent the remnant in the adult organs of the common biliopancreatic progenitors derived from the ventral endoderm of primitive duodenum (14). BTSCs can be easily isolated from adult or fetal organs, rapidly and clonally expand in culture and can differentiate toward several endoderm cell types including hepatocytes, cholangiocytes and pancreatic endocrine cells (17,24). A preliminary clinical report indicated the feasibility and safety of BTSC transplantation into the liver of patients with end-stage chronic liver disease (20). In general, the use of determined stem cells has the advantage of lower manipulation in comparison with reprogrammed cells and no need of immunosuppression in comparison with mature cells.

In conclusion, the development of novel strategies and the identification of novel cell sources should represent main efforts in the field of cell therapy for endodermal organs (Figure 1). Cell reprogramming strategies are optimal for establishing a stable cell source but safety issues should be addressed prior to translation in clinics. In this context, the study by Wang *et al.* strongly suggests that a preferable cell source for reprogramming would

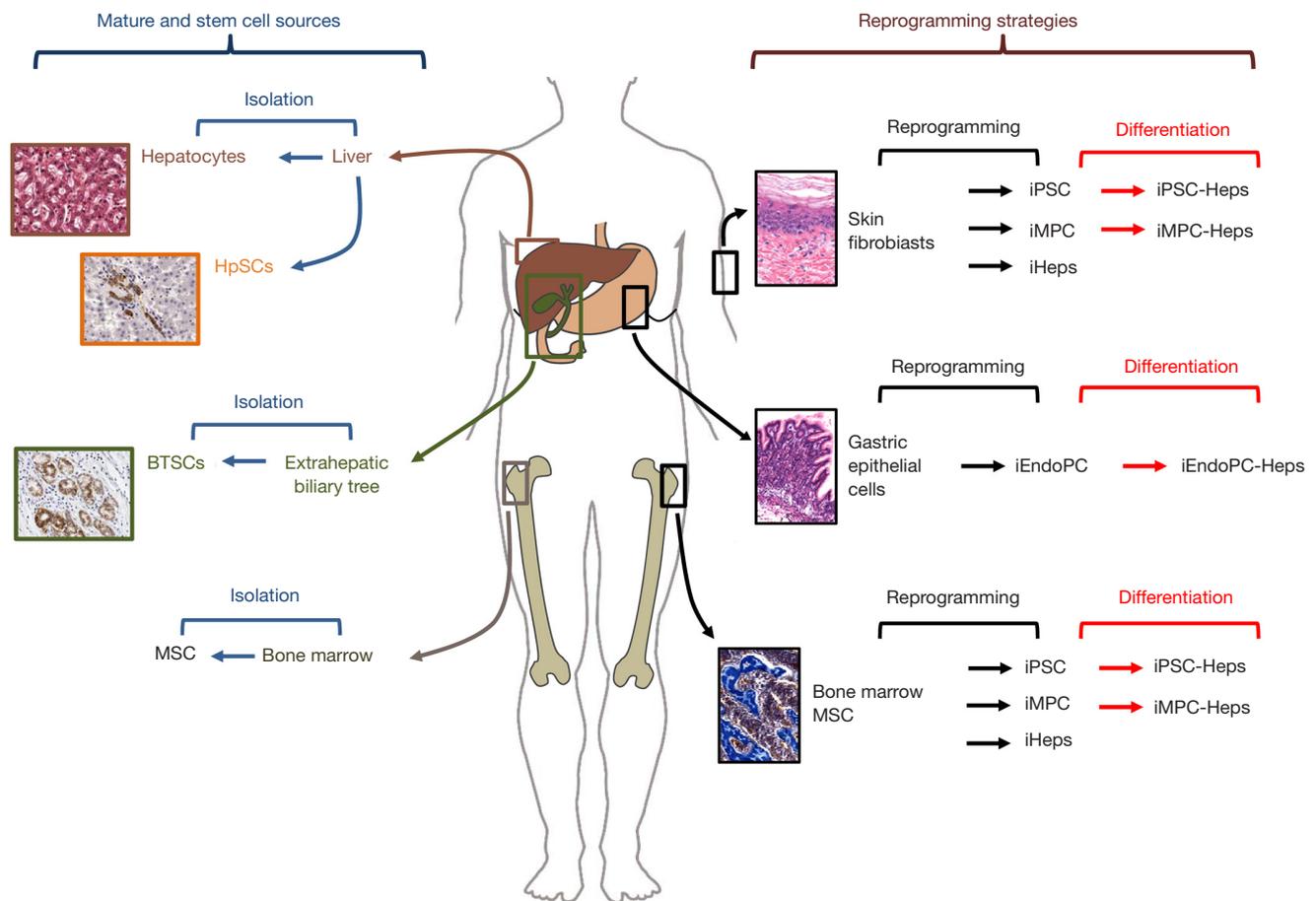


Figure 1 Possible cell sources for regenerative medicine of the liver. Possible sources for mature or stem/progenitor cells are indicated on the left. Possible sources and strategies for generating reprogrammed hepatocytes are reported on the right. BTSC, biliary tree stem/progenitor cells; HpSC, hepatic stem cell; Hep, hepatocytes; iEndoPC, induced endodermal progenitor cells; iHeps, induced Hepatocytes; iMPC, induced multipotent progenitor cell; iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cell.

be represented by organs with an endodermal origin (1); this approach would simplify protocols and reduce genetic manipulation; moreover, differentiation of reprogrammed cells towards mature endoderm cells would be facilitated since reprogrammed cells partly maintain an epigenetic memory of the starting cell type, leading to a preferential differentiation potential into the cell types they are derived from (25). On the other side, stem cell strategies should take in account the organ availability from donor organs or surgical procedures. However, the presence, phenotype and potentiality of a specific progenitor/stem cell population in adult organs are strongly associated with anatomical features and embryological development. Therefore, stem cells obtained from organs with an endodermal origin would be more prone to differentiate into mature endodermal

cells once transplanted and would be more adapt to clinical translation if compared with stem cells with a different embryological origin, such as MSCs.

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Footnote

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