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Cholangiocytes: Cell transplantation☆

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ABSTRACT

Background: Due to significant limitations to the access to orthotropic liver transplantation, cell therapies for liver diseases have gained large interest worldwide.
Scope of review: To revise current literature dealing with cell therapy for liver diseases. We discussed the advantages and pitfalls of the different cell sources tested so far in clinical trials and the rationale underlying the potential benefits of transplantation of human biliary tree stem cells (hBTSCs).
Major conclusions: Transplantation of adult hepatocytes showed transient benefits but requires immune-suppression that is a major pitfall in patients with advanced liver diseases. Mesenchymal stem cells and hematopoietic stem cells transplanted into patients with liver diseases are not able to replace resident hepatocytes but rather they target autoimmune or inflammatory processes into the liver. Stem cells isolated from fetal or adult liver have been recently proposed as alternative cell sources for advanced liver cirrhosis and metabolic liver disease. We demonstrated the presence of multipotent cells expressing a variety of endodermal stem cell markers in (peri)-biliary glands of bile ducts in fetal or adult human tissues, and in crypts of gallbladder epithelium. In the first cirrhotic patients treated in our center with biliary tree stem cell therapy, we registered no adverse event but significant benefits.
General significance: The biliary tree stem cell could represent the ideal cell source for the cell therapy of liver diseases. This article is part of a Special Issue entitled: Cholangiocytes in Health and Diseaseedited by Jesus Banales, Marco Marzioni, Nicholas LaRusso and Peter Jansen.

1. Introduction

Liver diseases represent a major public health problem affecting 5–15% of the inhabitants worldwide [1]. The final manifestation of chronic liver diseases is cirrhosis. When a successful etiologic approach is unavailable or has failed, or delayed, progressive, extensive fibrosis, with concurrently impaired hepatocyte regeneration, leads to irreversible cirrhosis and then liver failure. Currently, orthotropic liver transplantation (OLT) is the only therapy for acute and chronic liver diseases in the terminal stage. However, the number of donated livers is limited. In addition, OLT cannot be proposed for many cirrhotic patients, since at very advanced disease stages, surgical risks or other contraindications exist [2]. Post-surgery complications and rejection are still significant problems associated with organ transplantation. Other limitations of OLT relate to the high costs: typically, ~$150,000 to $180,000 for transplant and first-year medical follow-up [2]. These

Abbreviations: ALDH, Aldehydedehydrogenase; CD, Cluster differentiation; EGF, Epidermal growth factor; EpCAM, Epithelial cell adhesion molecule; ECM, Extracellular matrix; FGF, Fibroblast growth factor; GMP, Good manufacturing practice; G-CSF, Granulocyte colony-stimulating factor; HGF, Hepatocyte growth factor; hBTSCs, Human biliary tree stem cells; hHpSCs, Human hepatic stem/progenitor cells; HA, Hyaluronic acid or hyaluronan; INR, International normalized ratio; KLF, Kruppel-like factor; LGR5, Leucine-rich repeat containing G protein-coupled receptor 5; MSCs, Mesenchymal stem cells; MELD, Model of end-stage liver disease; NANOG, Nanog homeobox; NASH, Non-alcoholic steato-hepatitis; OCT4, Octamer-binding transcription factor 4; OLT, Orthotopic liver transplantation; PDX1, Pancreatic and duodenal homeobox 1; PBGs, Peri-biliary glands; PEG-DA, Polyethylene glycol-diacrylate; RT-qPCR, Quantitative reverse-transcription polymerase chain reaction; SALL4, Sal-like protein 4; SCID, Severe combined immunodeficiency; SOX, Sry-related HMG box

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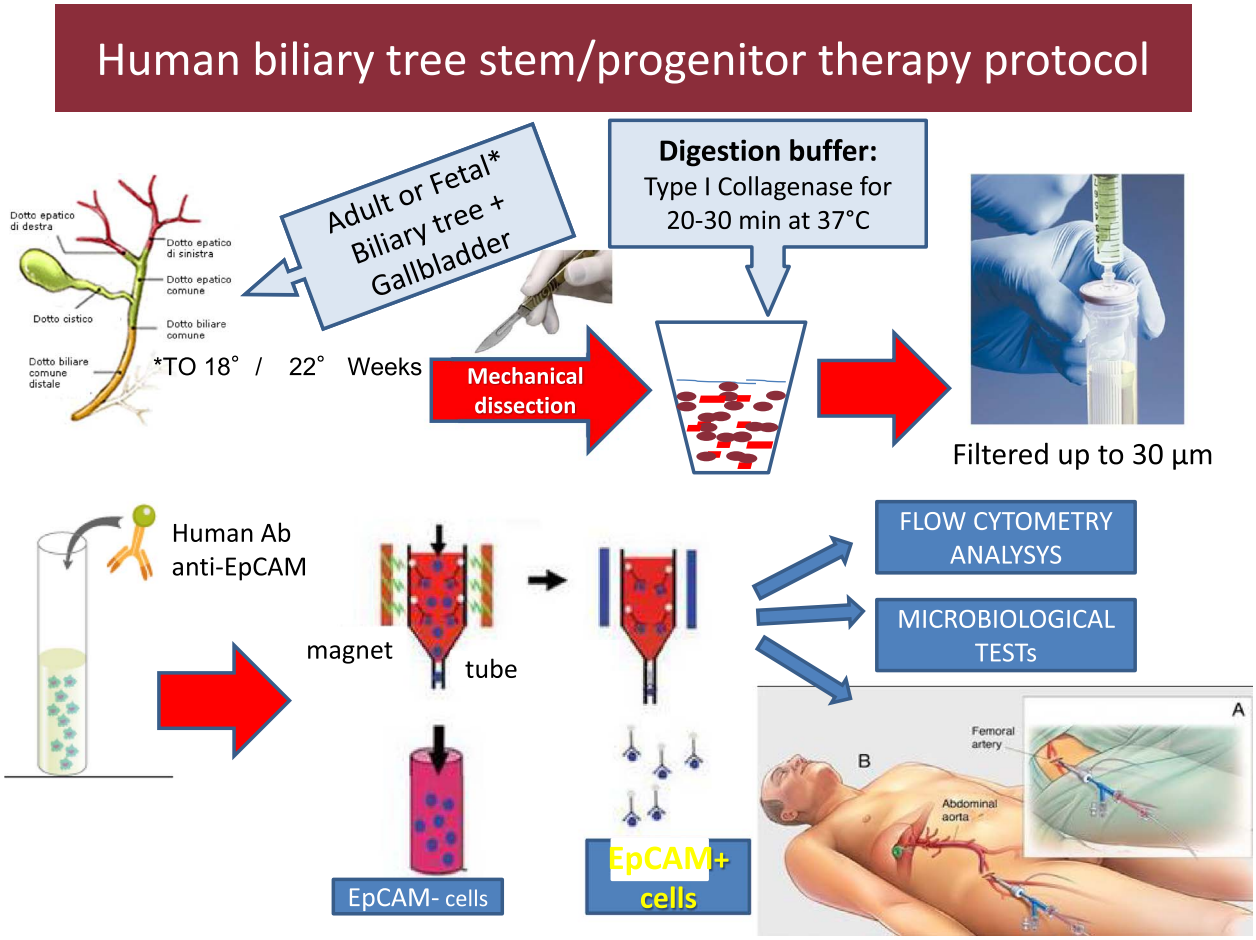

Fig. 1. Human biliary tree stem/progenitor therapy protocol. The isolation protocol involves either the biliary tree from livers of therapeutic abortions or the extrahepatic biliary tree from donors which are digested mechanically and enzymatically to obtain a cell suspension which is filtered and then subjected to cell immune sorting using EpCAM microbeads. The EpCAM sorted cells immediately underwent identity test by flow cytometry analysis and microbiological tests before sudden injection into the hepatic artery of patients who have been admitted to the transplant unit few hours in advance. TO: therapeutic abortion. EpCAM: epithelial adhesion molecule.

limitations drive interests to explore cell therapies aimed to sustain liver function, to stimulate liver regeneration and to reduce the scarring process.

2. Cell sources for cell therapy of liver diseases

Different strategies of cell therapy have been so far attempted and used not only for advanced cirrhosis but also for acute liver failure, fulminant hepatitis, inborn errors of metabolism, viral hepatitis, liver toxins, alcohol consumption, chronic cholestatic diseases, autoimmunity and metabolic disorders such as non-alcoholic steato-hepatitis (NASH) [3].

2.1. Adult hepatocytes

The first strategies tried for cell therapies of liver diseases focused on adult hepatocyte transplantation which was first used for patients with liver diseases in the 1990s and has continued through for the last ~20 years [4]. The studies have not expanded into large clinical trials due to difficulties in obtaining high quality donor cells, limitations in the ability to cryopreserve hepatocytes, and the complications that can occur with transplantation of the hepatocytes into the liver [4]. These complications include low engraftment (typically only 5% if transplanted via the portal vein), similar engraftment but with bleeding disorders if transplanted by direct injection, distribution to ectopic sites (most commonly the lungs), and formation of emboli that can be life threatening especially if they occur in the lungs [4]. Patients have been transplanted via the portal vein with isolated hepatocytes at concentrations from millions to billions. Immunosuppression was required in all cases. Although, it is a challenge to draw conclusions from the published reports, treatments resulted in improved liver functions, but the patient numbers in each trial are so small that they constitute only anecdotal evidence of effectiveness [4]. No trials have been done with sufficient numbers of patients to permit statistical analyses yielding Model of End-stage Liver Disease (MELD) or Child-Pugh scores, the standardized metrics used for liver condition. Therapeutic effects were observed to last from days to a few months and can provide bridging to a liver transplant [4]. Therapeutic benefits for congenital defects (e.g. Crigler-Najjar) [5–8] have lasted longer, typically for months and, in a few cases, up to a few years [8,9]. Leaders in the field have lobbied for alternate approaches but still retaining a focus on using adult hepatocytes [10], but the poor to mediocre results to date have diminished enthusiasm for this strategy.

2.2. Mesenchymal stem cells and hematopoietic stem cells

Transplantation of mesenchymal stem cells (MSCs) and hematopoietic stem cells into patients with liver disease, has resulted in large numbers of clinical trials throughout the world [11]. At present over 20 clinical trials have been published but with small numbers of patients (typically under 10). Thus, they are similar to the clinical trials of hepatocyte transplantation in providing anecdotal evidence or evidence with minimal possibility of statistically validated findings of the efficacy of the treatments. There are a small number with larger patient

Box 1
Factors potentially affecting the liver engraftment of transplanted cells.

Host factors

- Host liver extracellular matrix:
  - major driver of adhesion molecule expression
- Host liver pathologic conditions and related alterations of vasculature perfusion:
  - liver engraftment of hHpSCs or hBTSCs increases in the mouse model of CCl4-induced liver fibrosis with respect to normal liver [23,16]
  - engraftment and repopulation of transplanted cells have been demonstrated in genetic mice models of liver repopulation, after acute toxic liver injuries or in mice treated with compounds (retrorsine and monocrotaline) blocking hepatocyte proliferation. These models provide space for the growth of engrafted cells or a selective advantage for transplanted cells [58]

Factors related to the cell source

- Cell size
- Proliferation:
  - hBTSCs have intrinsic proliferative advantages [15,16]; the percentage of hepatic stem cells expressing proliferation markers was on average 45 times greater than the percentage of mature hepatocytes [31]
- Immunogenicity issues:
  - fetal hepatic and biliary stem/progenitor cells express no or minimal class I and II MHC antigens (reviewed in [25])
  - hBTSCs modulated T-cell apoptosis through the Fas/Fas ligand pathway [25]
- Tolerance to toxins and ischemia:
  - high expression of multidrug resistance proteins in stem cells (reviewed in [20] and [21])

Administration routes

- Hepatic delivery:
  - via portal vein or hepatic artery or direct injection into the liver
- Ectopic implantation:
  - into the peritoneum or into the spleen

Cell rejection: immunomediated cell rejection.
Abbreviations: hHpSCs, human hepatic stem/progenitor cells; hBTSCs, human biliary stem/progenitor cells; CCL4, carbon tetrachloride; MHC, major histocompatibility complex.

populations such as one reported by Peng et al. [11] involving 53 patients who underwent a single transplantation with autologous MSCs by a vascular route via the peripheral vasculature, the spleen or through the hepatic artery into the liver. The trials comprised treatments with: a) unfractionated bone marrow or peripheral blood; b) used cytokines (e.g. granulocyte colony-stimulating factor [G-CSF]) to mobilize cells in the bone marrow; c) used immune-selected cell populations (CD44 + cells, CD133 + cells) from bone marrow; or d) used cultured MSCs or cultures treated with growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF) or fibroblast growth factor (FGF). Transplantations of any of these forms of MSCs or hematopoietic stem cells were found to be safe and significantly improved the quality of life and liver functions [3]. The patient response occurred within days to weeks, but long-term effects (more than a few months) were not observed. The conclusions are that effects are due to trophic and immune-modulatory factors. Finally, a single specific molecule secreted by the MSCs was discovered, the Milk Fat Globule-EGF Factor 8, that protects against liver fibrosis in mice [12]. Andreone P. et al. [13] performed a phase I study where 12 patients with end stage liver disease were reinfused with highly purified CD133 + bone-marrow derived hematopoietic stem cells, after pretreatment with growth factors. No severe adverse events were registered; MELD worsened during mobilization in Child Turcotte-C patients. Improvement of liver function was observed 2 months after reinfusion (MELD 19.5 vs 16; $p = 0.045$), 5 patients underwent liver transplantation within 12 months from reinfusion and 2 died because of progressive liver failure. Again, the benefits were only transient and after a 6-month follow-up, liver function parameters returned to baseline. A recent multicenter phase-II open-label controlled trial of repeated autologous infusions of G-CSF mobilized CD133 + bone marrow stem cells in 27 patients with advanced cirrhosis (versus conservative management or treatment with G-CSF alone) found no impact on liver function nor on fibrosis and the trial was interrupted [14]. Therefore, also G-CSF and purified hematopoietic stem cells did not have an impact on liver function or fibrosis, according this large, powered, randomized controlled trial [14].

3. Human biliary tree stem cells

In a series of published studies [15–19] and summarized in recent reviews [20–22], we demonstrated the presence of cells expressing a variety of endodermal stem cell markers in (peri)-biliary glands (PBGs) of bile ducts in fetal or adult human tissues. We also found them in crypts of gallbladder epithelium [16]. The observations in situ in human biliary tree tissue have been complemented by demonstrations of culture selection of colonies of stem cells and that expand for months through self-renewal processes, and are multipotent as indicated by their ability to lineage restrict to hepatocytes, cholangiocytes or pancreatic islet cells depending on the precise conditions to which they are subjected. Isolation of major subpopulations of hBTSCs from tissues of all donor ages can be achieved by immunoselection for cells positive for expression of epithelial cell adhesion molecule (EpCAM). The hBTSCs constitute approximately 2–4% of the biliary tree cells with the percentages varying, depending on which part of the biliary tree is being analyzed. The richest sites are the large, intrahepatic bile ducts and the hepato-pancreatic common duct. The hBTSC subpopulations are all

Box 2
Strategies adopted to increase liver engraftment and repopulation.

Host pre-conditioning (clinical and pre-clinical evidences obtained in hepatocyte transplantation):

- Preparative hepatic irradiation in human and animal models [56]
- Hepatic resection in human and animal models [59]
- Portal vein embolization in animal models [60,61]

Use of matrix components and grafting strategies (pre-clinical studies):

- Preparative injection of fibrin in animal models of hepatocyte transplantation improves the engraftment [32]
- Bio-matrix scaffold components increase proliferation and differentiation of human hepatic stem/progenitor cells in vitro [62]
- Hyaluronic acid-coating increases the engraftment of human biliary tree stem cells in mice w/o damage when injected by vascular route into portal vein [47]
- Grafting strategies have been devised for transplantation of human hepatic stem cells (hHpSCs) embedded into a mix of soluble signals and extracellular matrix biomaterials (hyaluronans, type III collagen, laminin): grafted cells remained localized into the livers, resulting in a larger bolus of engrafted cells in the host livers, with respect to transplantation by direct injection or via a vascular route, under quiescent conditions and with potential for more rapid expansion under conditions of liver injury [46]
- Combination strategies:
  - advanced grafting devices directly transplanted;
  - liver scaffold transplantation [63]

Cotransplantation (pre-clinical stages):

- Some evidence exist that cotransplantation of stromal cells facilitates the process of hepatocyte engraftment and proliferation most likely by the release of cytokines and growth factors in mice [64]
- Pietrosi and co-workers treated 9 patients affected by end-stage liver disease with intrasplenic infusion of a total cell population obtained from the fetal liver, containing cells expressing typical MSC and HSC markers and found that the procedure was safe and well tolerated with positive effects on clinical scores and on encephalopathy [28]

Cell sources

- Stem cell therapies of liver diseases with hepatic and biliary stem/progenitor cells are based on favorable biologic properties of stem cells would improve cell engraftment and repopulation, and attenuate immunoreaction (see refs. [27–29]). However, no clinical trial comparing different cell types in humans is available.
- In a competitive liver repopulation mouse model (heterozygous alb-uPAtg1/2 mice), engraftment and repopulation capacities of mature hepatocytes were always superior to early and late hepatoblasts as well as embryonic stem (ES)- and iPS cell-derived cells. Any of the tested postnatal non-hepatocyte cells, such as hematopoietic stem cells or mesenchymal stromal cells, did not engraft long term and did not repopulate the liver [58]

Administration route:

- No trial exists in animal models comparing administration via portal vein versus hepatic artery. The former is mainly used for hepatocyte transplantation, while the latter for hepatic or biliary tree/stem cell transplantation
- Direct injection versus vascular route: directly injected hHpSCs embedded in bio-matrices remained localized into the livers, resulting in a larger bolus of engrafted cells in the host with respect to transplantation via vascular route [46]

Cell rejection:

- Pharmacology regimens limit immune rejection of human hepatocytes.
- Hepatocytes microencapsulated in alginate limit immunoreaction thanks to the limited access of transplanted cells to host immune cells [65]
- The use of fetal or adult hepatic and biliary stem/progenitor cells alleviated the immunogenicity issues since these cells express no or minimal class I and II MHC antigens, and since hBTSCs modulated T-cell apoptosis through the Fas/Fas ligand pathway (reviewed in [25]). No immunosuppression has been required in clinical trials [27,29]

Abbreviations: hHpSCs, human hepatic stem/progenitor cells; hBTSCs, human biliary stem/progenitor cells; CCL4, carbon tetrachloride; MHC, major histocompatibility complex; MSC, mesenchymal stem cell; HSC, hematopoietic stem cells, ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells.

small (7–9 μm), approximately half the size of mature parenchymal cells; express various stem cell markers such as endodermal transcription factors (SOX9, SOX17, PDX1), pluripotency genes (OCT4, SOX2, NANOG, SALL4, KLF4/5), CD133 (prominin), CD44 (hyaluronan receptors), aldehydedehydrogenase (ALDH), and hedgehog proteins and no markers of mature hepatic or pancreatic cells. They are also tolerant to ischemia [22]. Preliminary studies indicated that we can isolate viable and healthy hBTSCs from biliary tree tissue even 48 h or

more after cardiac arrest of the donor [23]. In addition, other preliminary studies suggest that the hBTSCs can be cryopreserved under serum-free, wholly defined conditions that we established and found effective for human hepatic stem cells [24].

In preclinical studies, we demonstrated that hBTSCs isolated from human gallbladder and transplanted in a model of liver cirrhosis yielded the formation of human hepatocytes and cholangiocytes and, mostly important, the improvement of the liver function tests in the model [15].

The hBTSCs can be isolated from fetal or adult livers or from adult gallbladder. hBTSCs are an ideal source of multipotent endodermal stem cells with relevant self-renewal and differentiative capacities. In fact, first, tissues are largely available since fetal biliary trees can be easily obtained from the fetal liver given the large number of therapeutic abortions, postnatal biliary tree tissues are largely available since most of the biliary tree is routinely discarded from liver and pancreatic transplants and gallbladder tissue is readily available given large numbers of cholecystectomy done routinely. Second, the hBTSCs can be isolated easily from the biliary tree of any donor age and survive and expand ex vivo under wholly defined and serum-free conditions that we established; v) hBTSCs from fetal tissue are non- or minimally immunogenic given their low or null expression of HLA class I and II [25] antigens and, therefore, can be transplanted without the need for immunosuppression; vi) hBTSCs, transplanted into experimental models of liver cirrhosis, are able to engraft, to differentiate into adult hepatocytes and cholangiocytes and to rescue liver function. The use of stem/progenitor cells has advantages over transplantation of mature hepatocytes. Firstly, stem/progenitor cells, capable of generating mature liver cells, can circumvent the shortage of hepatocytes. The hBTSCs are available from hepatic and biliary tree tissues of donors of all ages and can self-replicate under particular conditions indefinitely. Secondly, transplanting stem/progenitor cells may, in theory, yield better long-term repopulation and persistent metabolic activity due to the constant generation of newly formed mature parenchymal cells. Thirdly, stem/progenitor cells are not immunogenic [23,26] are more resistant than adult cells to ischemic damage, and are easier to cryopreserve [24]. All of these properties enable stem/progenitors to be a more robust product for clinical programs.

Khan et al. [27] isolated EpCAM + cell from the fetal livers and administered these cells into 25 cirrhotic patients via hepatic artery and without immunosuppression. The procedure was safe and associated with significant improvement of liver function that lasted during the six-month follow up period. This procedure was proven to be safe, as assessed by ultrasound indicating persistence of echotexture, no focal lesions, and no abnormal changes in the size of the hepatic artery. Fetal liver-derived EpCAM + cells (a mix of human hepatic stem cells/hHpSCs and hepatoblasts) were marked with Tc99m-hexamethylpropyleneamine oxime and injected; most of the marked cells remained within the liver lobe. Also, most patients had grade 2 to grade 3 esophageal varices before the transplants, and the majority showed reduction in the varices grading. More recently, Pietrosi and co-workers [28] treated 9 patients affected by end-stage liver disease within transsplenic infusion of a total cell population obtained from the fetal liver and found that the procedure was safe and well tolerated with positive effects on clinical scores and on encephalopathy.

With all these premises, representing a strong rational basis for translating our preclinical research into a clinical setting, after having obtained the required authorization, two patients with advanced cirrhosis were treated with fetal hBTSC transplantation via hepatic artery infusion (Fig. 1) [29]. We injected immunoselected EpCAM-positive cells which also co-expressed Leucine-rich repeat containing G protein-coupled Receptor 5 (LGR5). In fetal livers, LGR5-positivecells were located in the ductal plate and in the epithelium of larger bile ducts [18]. In the gallbladder and hepatic common duct, surface epithelial cells and bud of PBGs, were diffusely positive for LGR5 [17]. Cell products were characterized by flow cytometry (FC) for EpCAM and LGR5 before transplantation (Fig. 1). Estimated cell viability by trypan blue exclusion was routinely higher than 95%. The immunomagnetic sorting resulted in an enrichment of EpCAM-positive cell population to 51% and contained cells with co-expression of EpCAM and LGR5 (48.4%). Immunosorted EpCAM-positive cells were suspended in 10% glucose solution, at a concentration of 1 million cells per ml. The cell suspension was infused into the hepatic artery at an infusion velocity of 200 ml/h (Fig. 1). The first patient who received, via the right hepatic artery, 42 million viable EpCAM-sorted cells, showed an evident increasing trend of serum albumin, associated with a parallel gradual and constant decrease of International Normalized Ration (INR). Before treatment, the patient received repeated hospitalizations, mostly for treatment of ascites through large volume paracentesis but their frequency and duration were significantly shortened by the cell therapy (1-day vs 5-days hospital stay pre-treatment). The need of paracentesis was abolished during the follow-up, with no request after cell therapy. The patient observed the same pharmacological treatment during the whole period of observation, and did not received albumin injection. Six months after receiving hBTSC transplantation, the Child-Pugh score decreased from C-12 to C-10 and the MELD score from 24 to 20, mainly due to improvement of coagulation and ascites.

The second patient is a Caucasian female affected by HCV-related cirrhosis. No side-effects related to the cell infusion were registered during the scheduled 6-month follow-up. Six months after the treatment, the patient displayed a consistent amelioration of liver function: Child-Pugh score improved from C-11 to B-8 and the MELD score from 21 to 16. After the 12th month of follow-up the patient continued to maintain a state of compensated liver cirrhosis. Notably, this patient experienced a gradual and constant amelioration of albumin value and coagulation (INR) along 12 months of follow-up. The patient did not receive intravenous albumin infusion. Exhaustive information on the different cell sources for cell therapy of liver diseases containing data on cell origin, number/size/characteristics of clinical trials, vantages/disadvantages, references, etc., have been included as supplementary tables (one for cell source) in an updated review published in Stem Cells by international experts including us and collaborators from Prof. Lola Reid and Prof. Luca Inverardi groups (see ref. 20).

4. Strategies to ameliorate the engraftment efficiency of hBTSCs into the liver (see Boxes 1 and 2)

Liver parenchymal repopulation with exogenous cells is a prerequisite for a successful cell therapy. Cell translocation from sinusoids into liver plates requires disruption of the sinusoidal endothelium and progressive proliferation of the transplanted cells [30] (Box 1). BTSCs and hepatic stem/progenitor cells (HpSCs) have an intrinsic proliferative advantage when transplanted in liver parenchyma with respect to mature hepatocytes [31] (Box 1). However, the main pitfall of liver stem cell therapy is the uncertain yield of transplanted cells effectively grafting in the target organ. Indeed, engraftment efficiencies of less than 5% were reported for stem cells when delivered by vascular routes into the livers of primates [32] or in the livers of humans when injected into the portal vein [33]. That's because the modulation of cell adhesion and engraftment are key issues in regenerative medicine (Box 1). To this regard, a lot of recent researches focus on strategies to improve grafting efficiency of transplanted cells (Box 2). Hyaluronic acid (HA) is one of the most utilized biomatrix in human medicine and it could be one of the best candidates to promote the acceptance of transplanted cells in the target organs (Box 2). In the last decades, different evidences showed how extracellular matrix (ECM) plays a fundamental dynamic role in regulating cell homeostasis. Among the main ECM components, HA plays a major role because of its biologic, rheological, viscoelastic and hygroscopic properties [34]. HA molecules are highly active in interacting each other by forming large polymers or in association with other molecules [35]. HA is expressed on the cell surface of both normal and tumor cells, it is an important component of

the stem cell niche involved in preserving the multipotent character of stem/progenitor cells, preventing their differentiation and modulating stem cell migration during embryonic development [36]. Indeed, the HA matrix supports cell adhesion, growth and differentiation, regulates cell trafficking and affects various biologic processes including development and organogenesis, inflammation, wound healing, and tissue remodeling [34]. For this reason, many research groups used scaffold/gel with HA, alone or with other molecules [37–44]. Lozoya et al. [43] studied the grafting of hHpSC using hydrogel in several concentrations with thiol-modified HA derivatives, and polyethylene glycol-diacrylate (PEG-DA), as cross-link. Remarkably, they demonstrate that both the composition and mechanical properties of the microenvironment can regulate phenotypic changes. Based on these results obtained in vitro, Reid L. et al. [46] devised grafting strategies for transplantation of hHpSCs embedded into a mix of soluble signals and extracellular matrix biomaterials (hyaluronans, type III collagen, laminin) found in stem cell niches. Grafted cells remained localized to the livers, resulting in a larger bolus of engrafted cells in the host livers under quiescent conditions and with potential for more rapid expansion under injured liver conditions. By contrast, transplantation by direct injection or via a vascular route resulted in inefficient engraftment and cell spreading to ectopic sites (Box 2). Recently, we developed a fast and easy method to coat hBTSCs with HA and assessed the effects of HA-coating on cell properties in vitro and in vivo [47] (Box 2). The HA coating markedly improved the viability, colony formation, and population doubling of hBTSCs in primary cultures, and resulted in a higher expression of integrins that mediate cell attachment to matrix components. When HA-coated hBTSCs were transplanted via the spleen into the liver of immunocompromised mice, the engraftment efficiency increased to 11% with respect to 3% of uncoated cells (Box 2). Studies in distant organs showed minimal ectopic cell distribution without differences between HA-coated and uncoated hBTSCs and, specifically, cell seeding in the kidney was excluded. The danger of thrombi generation and ectopic distribution has been minimized, because the coating process did not result in large cell clusters. Moreover, the fact that HA is selectively and actively cleared by the liver could be an additive factor favoring the selective hepatic engraftment [48]. Finally, HA should create a favorable microenvironment allowing a greater number of hBTSCs to engraft within the liver parenchyma, survive, proliferate, and enhance human albumin secretion. When, in previous studies, fibrin was tested, instead of HA, a similar phenomenon was observed [32]. Previous studies highlighted varied HA trophic effects in different cell systems [49–54]. An especially advantageous aspect of using HAs to facilitate engraftment in condition of liver injury is that they are anti-inflammatory and entirely biocompatible. In prior studies with the use of HAs for diverse forms of transplantations, they minimize fibrotic reactions and foster vascularization that facilitates engraftment [55]. HA is a natural candidate for stem cell grafting because it is abundant during embryogenesis, wound repair, and organ regeneration [45]. With regard to the latter, HA cell coating can be seen as a form of host conditioning, and it is currently a target to monitor cell rejection in liver cell therapy in humans [56]. HA coating could improve outcomes of stem cell therapies of liver diseases and could be immediately translated into the clinic given that Good Manufactory Practice (GMP)-grade HAs are already available for clinical use. This molecule indeed is already approved for clinical use and has been tested in clinical trials [57].

5. Conclusions and perspectives

The improvement of regenerative medicine approaches for liver diseases requires the identification of sustainable and readily available cell sources [66]. Other than mature hepatocytes and the different stem cells already tested in clinics, the possibility of reprogramming adult somatic cells to generate mature liver cells is attractive [66]. As far as biliary tissue is concerned, several groups have reportedly defined protocols to differentiate human induced pluripotent stem cells (iPSCs) in functional biliary epithelium cells [67–71]. This technology associated with dedicated tissue engineering strategies could lead to the autologous replacement of a damaged biliary tree [67–71]. However, the clinical application of reprogrammed cells raises concerns due to the possible uncontrolled tumorigenic expansion within the recipient, particularly, in long life expectancy situations as in pediatric liver diseases or in chronic liver diseases [66]. In this context, the small molecule-based reprogramming has raised attention due to its safety [66], and a recent paper by Wang et al. described a novel approach to generate human induced endodermal progenitor cells (hiEndoPCs) through the lineage reprogramming of gastrointestinal epithelial cells induced by a cocktail of defined small molecules in association with the support of tissue-specific mesenchymal feeders [72].

Waiting for protocols allowing a safe and effective clinical translation of iPSCs in regenerative medicine of liver, hBTSCs represent a ready cellular source. The rationale of “hBTSC treatment of liver cirrhosis” is based on these key concepts: i) autochthonous hBTSCs are normally involved in liver repair but they are impaired in their regeneration potential and in their capacity to differentiate in mature liver cells, by the long-lasting inflammatory milieu characterizing liver cirrhosis; ii) in preclinical studies we demonstrated that isolated fetal hBTSCs are capable of generating in vitro mature liver cells and that; iii) hBTSCs, transplanted in a model of liver cirrhosis, yielded the formation of human liver cells (hepatocytes and cholangiocytes) and, mostly important, the improvement of the liver function tests in the model, iv) hBTSCs are not immunogenic and are more resistant than adult cells to ischemic damage; v) in the first two cirrhotic patients treated in our center, we registered no adverse event but significant benefits. With these premises, the expectancy is that hBTSC transplantation in patients with advanced liver cirrhosis may yield long-term repopulation and persistent metabolic activity due to the constant generation of newly formed mature parenchymal cells and that this could finally result in improvement of clinically relevant liver functions.

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Conflicts of interest

V. C., G. C., E. G., and D. A. are inventors of international patents related to hBTSCs and were granted a sponsored research agreement from Vesta Therapeutics (Bethesda, MD) for further development of this patent.

Transparency Document

The Transparency document associated with this article can be found, in online version.

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