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New horizons for stem cell therapy in liver disease

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Summary

There is an increasing range of potential applications of stem cells in liver diseases, with many clinical studies already undertaken. We identify four of the main areas which we propose stem cell therapy could be a realistic aim for in the future: (1) to improve regeneration and reduce scarring in liver cirrhosis by modulating the liver's own regenerative processes, (2) to down-regulate immune mediated liver damage, (3) supplying hepatocyte-like cells (HLCs) derived from stem cells for use in extracorporeal bio-artificial liver machines, and (4) to use stem cell derived HLCs for cell transplantation to supplement or replace hepatocyte function.

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Whilst there have been advances in our understanding of the role of stem cells in liver damage and repair as well as encouraging results using stem cells as cell therapy in pre-clinical animal models, the precise mode of action and optimal cell usage has not been completely defined. Moreover, clarity is required as to what effects are needed in different types and severity of liver disease (Fig. 1). Nonetheless, clinical trials of autologous cell therapy for liver disease have begun. Small scale cell therapy studies with autologous adult stem cells have demonstrated safety and suggested possible benefit [1]. This has driven the development of larger studies to more rigorously test these observations. It is clear that stem cells and their progeny have a variety of putative functional roles, requiring careful thought as to what biological action is intended after their infusion. For example, mesenchymal stem cells (MSCs) have immunomodulatory capacity and cells of the haematopoietic lineage may have anti-fibrotic and pro-regenerative effects, suggesting that the choice of therapeutic cell may need to be tailored to the type of liver disease targeted as the required therapeutic effect may be very different.

Stem cells for the treatment of liver disease

The mismatch between the number of patients requiring transplantation for end stage liver disease and the number of available organs is set to grow, highlighting the need to develop new strategies to stimulate liver regeneration and reduce liver scarring. Recent work suggests that these two aims are inextricably linked, and that reducing hepatic fibrosis can result in activation of hepatic progenitor cells (HPCs) resulting in parenchymal regeneration [2].

Degradation of excess liver scar is thus a suitable target for cell therapy, and in this regard, Sakaida *et al.* have shown in a mouse model of liver fibrosis that autologous bone marrow cells (BMCs) injected via the tail vein can engraft the liver and are able to reduce liver scarring as well as stimulating this regenerative process [3]. In these studies, it was suggested that murine Liv8$^-$ non-haematopoietic cells were responsible for this effect [3], although the identity of these cells is not entirely clear. Furthermore, defining the exact nature of these cells in the human setting would be important to develop this approach as a human therapy. More compelling evidence for the action of human haematopoietic stem cells (HSCs) comes from their use in patients with liver cancer (mainly metastasis) and otherwise normal liver parenchyma [4]. Furst *et al.* included patients for whom [4] resection of the liver cancer was not possible at the outset as the residual liver volume would be insufficient for the patient to survive. Autologous bone marrow CD133$^+$ cells were selectively infused into the non-occluded segments II and III portal branches 2–4 h after portal vein branch embolisation (I, IV, V–VIII); to see if this would stimulate liver regeneration thus allowing earlier resection of the tumour. Liver volume increased much quicker in patients that received stem cells, such that cancer resection could be undertaken much sooner (27 days ± 11 *vs.* 45 days ± 21, p = 0.6). It should be noted however that this was a small study and whilst there was a control arm it was not a randomised trial, and importantly none of the patients had intrinsic liver disease. Furthermore, as with many human studies, the mechanism of action was not explored.

Macrophages, cells of haematopoietic origin, are known to play a critical role in regulating liver fibrosis in murine models [5]. A single intraportal administration of macrophages has

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Abbreviations: ECM, extra-cellular matrix; EPCs, endothelial progenitor cells; HLCs, hepatocyte-like cells; HPC, hepatic progenitor cell; iPSCs, induced pluripotent stem cells; MMP, matrix metalloproteinase; MSCs, mesenchymal stem cells.

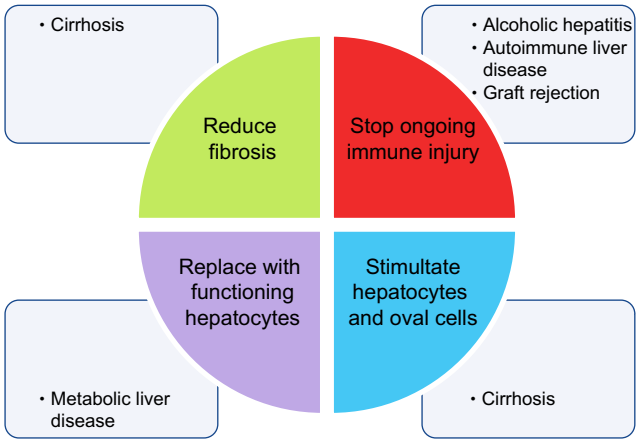

Fig. 1. Requirements for different types of liver injury. This figure illustrates the requirements that exist in differing types of liver injury.

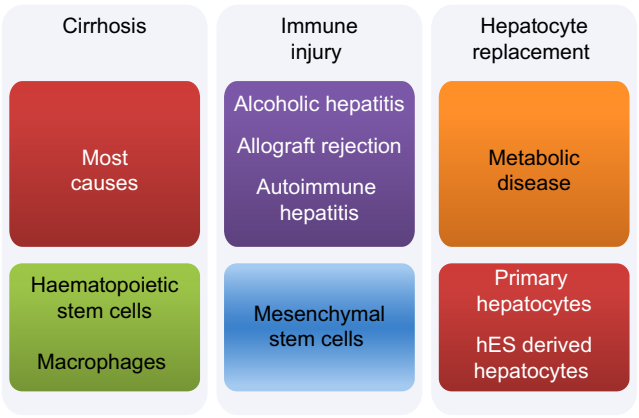

Fig. 2. Tailoring cell therapy. This figure suggests possible tailored cell therapy options for the major types of liver disease.

recently been shown to reduce fibrosis in a murine model of liver injury and increase regeneration [6]. Interestingly, the macrophages may have both direct and indirect effects upon the damaged liver as cell administration triggered the recruitment of endogenous inflammatory cells to hepatic scar areas and potentially amplified the donor cell effect [6]. This "cell amplification effect" does hold promise for clinical cell therapy where donor cell numbers may be limited. Epithelial progenitor cells have also been used successfully to reduce fibrosis in a rodent model of liver cirrhosis, however, it may be difficult clinically to isolate a source of endothelial progenitor cells for this purpose in humans [7].

There is some concern regarding the use of autologous unsorted BMCs as an injectable "therapy" for liver cirrhosis as the BMCs contain MSCs, cells that can differentiate into myofibroblasts, the scar forming cells of the liver [8] in certain settings. Of note, recent data showed that use of whole bone marrow as cell therapy in a rodent model of chronic liver injury leads to a worsening of liver fibrosis [6]. Uncontrolled clinical studies using infusions of unsorted autologous BM-derived mononuclear cells infusions for liver cirrhosis have been reported to show a reduction in Child's Pugh score and liver scarring and increased hepatocyte proliferation [9]. This may reflect the absence of stromal cells in mononuclear cell preparations, but also highlights the possibility that there may be differences between human and murine responses to cell therapy. This will be challenging as use of novel cell populations or combinations in patients will generally be informed by rodent studies.

MSCs have been reported to contribute to the direct production of new hepatocytes as well as to stimulate proliferation of endogenous hepatocytes [10,11], although this is not a universal finding [12]. Furthermore, the description of such "hepatocyte-like cells" is often incomplete, and does not as yet represent their adoption of a majority of a hepatocyte's complex phenotype by the MSC or their progeny [13]. To demonstrate that a MSC (or other stem cell) had differentiated into a hepatocyte would require the demonstration of hepatocytic functionality *in vitro* and *in vivo* for robust confirmation. It is therefore possible that much of the impact of injected MSCs in liver injury models may not relate to their adoption of a hepatocyte phenotype, but through other mechanisms. The immunomodulatory properties of MSCs have been demonstrated in a range of rodent models of non-hepatic [14] and hepatic [15] immune-mediated injury, as well as clinical studies in patients with GVHD who have hepatic damage where clinical benefit is reported [16]. Protocols for the isolation and characterisation of MSCs are evolving, and it is likely that there are different functional sub-sets which may mediate anti- and pro-inflammatory actions [17]. Despite this ambiguity regarding the function of MSCs in the literature there have been clinical studies reported suggesting that cell therapy with MSCs for liver cirrhosis can be beneficial but it is important to note that these studies are small and uncontrolled so whilst interesting should be interpreted with caution [18].

Of the clinical studies published, the overwhelming data suggests stem cell therapy is safe [1], although there are possible concerns regarding the route of delivery of cell therapy. Whilst no studies report superior outcomes when cells are directly injected into the liver (portal vein or hepatic artery), there have been complications such as hepatic artery dissection [19] and increased portal hypertensive bleeding [20] following this approach. Furthermore, the intravenous administration of autologous BM mononuclear cells resulted in hepatic homing of the injected cells suggesting this easier, safer route may be an adequate option for cell delivery [9,21]. Assuming that delivery to the liver is important for stem cell infusions to exert their optimal effect, then developing a better understanding of the mechanisms regulating their hepatic ingress may allow for further improvements to treatment protocols.

Whilst patients with a wide range of disease severity have been included in clinical trials, the priority remains to irrefutably confirm the efficacy of cell/stem cell therapy. In this regard, choosing patients in which the benefit may be most reliably determined and of greatest value is important. Patients verging on the cusp of requiring a liver transplant (e.g. with MELD score approaching/just below 15) are good candidates as even a small percentage improvement in liver function may be sufficient to significantly delay or indeed remove altogether the need for liver transplant.

For patients with cirrhosis/advanced fibrosis, the data at present would support further studies with macrophages, HSCs and BM mononuclear cells, whereas the immunomodulatory/anti-inflammatory properties of MSCs require further confirmation in immune-mediated models of liver injury. Further data may allow for the tailoring of cell therapy towards specific types of liver injury (Fig. 2).

Stem cells as a source of hepatocyte like cells

There have been many reports that various adult stem cells have the capacity to differentiate into hepatocyte like cells, although most of these studies incompletely characterise the stem cell derived hepatocytes "hepatocytic functions" or do not demonstrate *in vivo* functionality to the same extent as endogenous hepatocytes [13]. Whilst there are examples of "hepatocyte-like cells" produced from non-hepatic adult stem cells [22], it is our view, however, that this is unlikely to be a significant source of new hepatocytes that are of sufficient functionality to be clinically relevant. The liver's own HPCs are a realistic potential source of hepatocytes, however thus far it has proven difficult to isolate and expand these cells from the human liver and then control their differentiation into hepatocytes of sufficient number and quality. Indeed, the liver's own HPCs may be best targeted *in situ* via cell therapy, drugs or other such small molecule approaches.

Embryonic stem cells have the advantage of being able to proliferate in an unlimited fashion and produce large numbers of HLCs in both mouse and man settings [23]. *In vitro*, these cells have been shown to have reasonable functional capacity, although there is still caution about their use for transplantation due to their propensity to form both malignant and non-malignant tumours. Further work is required to reduce this risk, which may involve more definitive hepatocytic differentiation of HLCs, use of highly sorted populations to exclude contaminating cells and incorporation of clinically approved suicide genes (http://www.lentigen.com/products/lg690). In addition, there are ethical issues regarding the use of human embryonic stem cells, which will always have implications for their clinical use.

A recent development allows for the production of similar cells, induced pluripotent stem cells (iPSCs), by over-expressing transcription factors such as SOX2 and Oct4 in adult somatic cells. Keratinocytes isolated from skin biopsies have been used as a starting cell population to produce these iPSCs. This technology has great potential for disease modelling as the cells can be readily obtained from patients with metabolic diseases, and the derived cells are likely to exhibit metabolic defects, thus allowing the development of "liver disease in a dish" studies [24]. Hepatocytes derived from iPS cells have reasonable synthetic and metabolic capacity [25], and seem to be similar to cells derived from ES cells [26,27]. However, the same concerns remain about their use in a transplant setting, as we cannot yet be certain that these cells would not undergo reversion to more primitive state with uncontrolled expansion within the recipient.

Conclusions

Cell therapy is an exciting but challenging frontier in Hepatology, offering the potential for a range of new therapeutic interventions. This reinforces the need to develop strategies to improve liver regeneration. In this regard, cells which modulate liver fibrosis, or which act directly on the liver's own HPC population seem likely candidates as do small molecules and drugs. In addition, iPSCs are excellent candidates for the production of HLCs although there is caution about their *in vivo* use until long term data has shown that these cells can behave in an appropriate homoeostatic manner and not develop into tumours.

Conflict of interest

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

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