BONE MARROW STEM CELLS AND LIVER DISEASE

RECENT ADVANCES IN BASIC SCIENCE

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Y N Kallis, M R Alison, S J Forbes

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Stem cells are present in a variety of organs including the bone marrow (BM). Their role is to replenish multiple mature differentiated cell types and thereby achieve long-term tissue reconstitution. Stem cells retain the capacity to generate progeny and renew themselves throughout life. Haematopoietic stem cells (HSCs) are the main stem cell population within the BM and give rise to all mature blood lineages via erythroid, myelomonocytic and lymphoid precursors. A second type of bone marrow stem cell (BMSC), the mesenchymal stem cell (MSC), forms stromal tissue and can give rise to cells of mesodermal origin.

A longstanding principle of cell biology has been that cell loss is reconstituted via stem cells resident within and specific to an organ. However, recent work suggests that this paradigm may not hold for all organs or all types of injury, and tissue damage may attract migratory stem cell populations, particularly those from the BM. This observation has caused considerable interest in the field of liver disease, where new strategies to restore hepatocyte number, augment liver function and counteract progressive organ fibrosis are required. This article will focus on the various relationships between BMSCs and liver disease. It will concentrate on the evidence from animal models and human studies that BMSCs may aid in the regeneration of liver cell populations and may also contribute to the pathogenesis of liver damage. It will discuss the potential to use BMSCs for therapeutic application and review the current status of clinical trials in patients with liver disorders.

BMSCS DO NOT CONTRIBUTE SIGNIFICANTLY TO HEPATIC PARENCHYMAL CELL REPOPULATION UNDER NORMAL CIRCUMSTANCES

The physiology of liver cell regeneration

The hepatic parenchyma is made up of hepatocytes and cholangiocytes. Unlike other organs such as the gut, liver cell mass is restored primarily through division of the majority of mature hepatocytes and not via a dedicated stem cell population. After a regenerative stimulus, such as a two-thirds partial hepatectomy, most hepatocytes rapidly enter the cell cycle and undergo symmetrical mitosis. Liver cell mass can be restored via an average of less than two cell division cycles, albeit individual hepatocytes seem to have an intrinsic capacity for up to 70 doublings in serial transplantation experiments.¹ At times of overwhelming cell loss, with longstanding iterative injury (eg, chronic viral hepatitis), or when hepatocyte replication is impeded (eg, replicative senescence of steatohepatitis), regeneration seems to occur via a second cell compartment.^{2,3} This compartment remains poorly defined and seems to arise from a less differentiated cell population within the terminal branches of the intralobular biliary tree—the canals of Hering.⁴ In rodents these cells are called oval cells, but in humans they are more aptly named hepatic progenitor cells.⁵ Attempts to identify the originating stem cell are hampered by a paucity of specific cell surface markers.

Do BMSCs become hepatocytes? Transdifferentiation or cell fusion

Initial studies in humans suggested that some hepatocytes have a BM origin. Using Y chromosome tracking, a sparse number of hepatocytes seemed to be originating from the BM in male recipients of female orthotopic liver transplants, and in females who had received bone marrow transplantation (BMT) from male donors and thereafter developed liver disease.^{6,7} Similarly, other epithelial tissues, such as gut and skin, seemed to harbour cells of BM origin.⁸ Investigators then turned to an animal

See end of article for authors' affiliations

Correspondence to:
Professor S J Forbes,
MRC/University of Edinburgh
Centre for Inflammation
Research, The Queen's Medical
Research Institute, 47 Little
France Crescent, Edinburgh
EH16 4TJ, UK; stuart.forbes@
ed.ac.uk

Abbreviations: BM, bone marrow; BMSC, bone marrow stem cell; BMT, bone marrow transplantation; CCl₄, carbon tetrachloride; EMT, epithelial mesenchymal transition; EPC, endothelial progenitor cell; FAH(–/–), fumarylacetoacetate hydrolase knockout mouse; G-CSF, granulocyte-colony stimulating factor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HSC, haematopoietic stem cell; HpSC, hepatic stellate cell; MAPC, multipotent adult progenitor cell; MMP-9, matrix metalloproteinase-9; MSC, mesenchymal stem cell; NOD/SCID, non-obese diabetes/severe combined immune deficiency; SDF-1, stromal derived factor-1

Figure 1 Derivation of bone marrow stem cell subpopulations. Mesenchymal stem cells (MSCs) and multipotent adult progenitor cells (MAPCs) derived through characterisation of adherent cell populations. Haematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs) obtained through flow cytometric cell sorting (FACS) according to cell surface markers (examples shown). Side population (SP) cells are present in the subpopulation as side scatter on FACS plot, owing to their ability to efflux Hoechst 33342 dye.

model of hereditary type I tryosinaemia, the fumarylacetoacetate hydrolase knockout mouse (FAH^{-/-}), in which it seemed that this potentially fatal enzyme deficiency could be rescued through repopulation of the abnormal liver by BM cells derived from wild-type donors. The implication was that stem cells could cross conventionally demarcated lineage boundaries through a process termed transdifferentiation or stem cell plasticity, leading researchers to question the long-held tenets of cell biology. With time, it became apparent that these initial observations were difficult to reproduce, and later elegant studies in the same FAH^{-/-} mouse model conclusively showed that monocyte–hepatocyte fusion was the explanation for the restored normal phenotype to the FAH-deficient liver, in which hepatocytes formed by fusion expanded rapidly owing to a considerable survival advantage.^{9–10}

Unfortunately, in the absence of a strong selective pressure, it seems that stable long-term engraftment of BM-derived parenchymal cells is unusual. In rats given inhibitors of hepatocyte replication (eg, d-galactosamine, retrorsine or 2-acetylaminofluorene), if subjected to a regenerative stimulus such as a partial hepatectomy, BM-derived oval cell engraftment can rapidly decrease with time to <1%.¹¹ In the hepatitis B surface antigen transgenic mouse, the BM contributed to hepatocyte repopulation through cell fusion, but only at a very modest rate. In this model, constitutive HBsAg expression induces chronic low-grade hepatocyte turnover with nodule formation, and inhibition of hepatocyte replication with retrorsine provokes an oval cell response. Here, the contribution from BM-derived cells to hepatocyte repopulation waned to just 1.6% by 6 months, presumably owing to lack of a sustained selection advantage.¹² Likewise, when human HSCs were transplanted into carbon tetrachloride (CCl₄)-damaged non-obese diabetes/severe

combined immune deficiency (NOD/SCID) mice, donor-derived hepatocytes expressing mRNA for human albumin and α -1 antitrypsin were found in the liver. These hepatocytes occurred through cell fusion, but the phenotype of the chimaeric cells was variable and donor-derived genetic material was lost over time.¹³ When human cord blood, a rich source of progenitor cells, was transplanted into sublethally irradiated NOD/SCID mice, a contribution to the hepatocyte population of only 0.01% was found in the undamaged liver, reportedly through transdifferentiation.¹⁴ However, a subsequent study using human cord blood cells again demonstrated only low levels of hepatocyte repopulation even after CCl₄-induced or hepatocyte growth factor (HGF)-induced regeneration. Here the cells were chimaeric for both human and mouse antigens, suggesting that cell fusion rather than transdifferentiation had occurred.¹⁵

The current balance of evidence therefore suggests that, under circumstances of severe or repeated injury, BM cells can contribute to only a minor amount of liver parenchymal regeneration, primarily through cell fusion.¹⁶ A continued substantial selection pressure is required to produce a stable, expanding cell population of any significant size, and even then it is not clear whether fully functional hepatocytes result from these processes. Moreover, monocyte–hepatocyte fusion leads to polyploidy and chromosomal instability—both recognised as potential pathways to carcinogenesis in the damaged liver. In therapeutic terms, cell fusion may be a powerful tool to correct metabolic disorders of hepatic origin. This has been exploited in a number of isolated clinical scenarios. For example, sequential healthy donor hepatocyte transplantation was able to moderate the clinical phenotype of argininosuccinate lyase deficiency, an inborn error of metabolism, in an affected child for periods of >1 year. Histological engraftment through cell fusion of over 10% was detectable

Figure 2 (A) Potential contribution of (HSC)-derived cells to the damaged liver. Hepatocyte regeneration from bone marrow (BM) is most likely to occur through cell fusion (2) with a myelomonocytic derivative; transdifferentiation (1) is less likely; macrophages may also contribute to degradation (3) of fibrous matrix during recovery phase by release of matrix metalloproteinases (MMP). Circulating fibrocytes can form collagen-matrix-producing cells (4) in the liver. (Sex chromosome complements from male into female BM transplantation experiment are shown for illustrative purposes.) (B) Potential contribution of mesenchymal stem cells (MSC) to damaged liver. The most dominant axis seems to be transdifferentiation to a collagen-producing myofibroblast cell population. It is not clear whether MSCs contribute to hepatocyte regeneration at all. (C) Potential contribution of endothelial progenitor cells (EPCs) to damaged liver. Vasculogenesis by differentiation into sinusoidal endothelial cells. Intrahepatic release of growth factors such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) may promote hepatocyte regeneration and inhibit the deposition of fibrous matrix.

together with an improvement in clinical and metabolic indices.¹⁷ BM-derived stem cell treatment has yet to be clinically implemented for these conditions, but BMSCs are relatively accessible, which may make them an appealing alternative.

Which BMSCs populate the liver?

The BM has more than one stem cell population (fig 1). HSCs give rise to erythroid, lymphoid and myelomonocytic lineages, and are defined by their ability to rescue a lethally irradiated host animal through reconstitution of all essential BM function. Located within the stroma, the MSC is defined by its potential for trilineage differentiation into adipocytes, chondrocytes and osteoblasts.

The evidence as to which type of BMSC is responsible for liver repopulation is conflicting (figs 2A,B). In early studies looking at BM contribution to hepatocytes in the FAH(−/−) mouse, it seemed that HSCs were the stem cell fraction involved.¹⁸ The HSCs seemed to be the key cell in BMT experiments of CCl₄ liver injury in irradiated C57/B6 mice.¹⁹ Here cell fusion again seemed to be the mechanism of BM cell engraftment to the liver, but rates were low, at 1 in 250 000, and could be enhanced by the co-administration of granulocyte-colony stimulating factor (G-CSF). In a study of similar design, again using G-CSF, a higher proportion of BM-derived hepatocytes was found, at 3.6%, but endogenous hepatocyte regeneration nonetheless predominated after CCl₄ injury.²⁰ The induction of genes associated with immature hepatocytes (such as α -fetoprotein) was shown in a subfraction of BM cells expressing typical HSC surface markers after liver damage caused by a partial hepatectomy and 2-acetylaminofluorene.²¹ Certainly, in vitro, HSCs can be induced to differentiate into hepatocyte-like cells, given the appropriate medium containing HGF. More importantly, when cocultured with injured hepatocytes across a barrier through which soluble mediators can pass, HSCs could be induced to differentiate into hepatocytes.²² Conversely, when

human BMSC fractions were directly xenografted into rat liver damaged with allyl alcohol, only the MSC fraction seemed to give rise to hepatocyte-like progeny, positive for mRNA albumin expression.²³ Certainly in vitro transdifferentiation of MSCs into hepatocytes can be demonstrated when co-cultured with foetal liver cells.²⁴ Whether it is the HSC or the MSC compartment that contributes to BM-derived hepatocytes, or whether it can be both, remains unresolved.

What about monocytes central to the fusion process? When human monocytes were treated with macrophage-colony stimulating factor and interleukin-3 and subsequently conditioned with hepatocyte medium, cells with the morphology, marker gene expression and metabolic function of hepatocytes were found. On transplantation into NOD/SCID mice, these cells showed liver integration and albumin expression.²⁵ Another more recently identified stem cell is the multipotent adult progenitor cell (MAPC). It is a primitive cell isolated from adult human and rodent BM tissue and seems to have an extensive capacity for proliferation and multilineage differentiation in vitro. Whether it has relevance in vivo is yet to be conclusively determined. One study has shown that rodent and human MAPCs can be induced to adopt a hepatocyte phenotype in vitro and can display limited hepatocyte function (eg, secrete urea, cytochrome P450 activity).²⁶ MAPCs can also apparently differentiate into hepatocytes when infused in vivo into non-irradiated mice, although function was not determined.²⁷ As a word of caution, it is worth noting that other laboratories have found it notoriously difficult to propagate MAPCs from BM.²⁸

What is becoming clear from observations in these BMT experiments is that much of the interpretation is dependent on precisely how cell populations are isolated and defined. Previously determined distinctions between HSCs and MSCs may become obsolete given the recognition that stem cell fractions can be very heterogeneous. To illustrate this, a

multipotent stem cell subpopulation with HSC-like properties but that is CD34-negative has recently been defined. This side population cell is classified by its ability to efflux Hoechst 33342 dye, leading to its identification as a dog leg-shaped “side population” on flow cytometric scatter analysis (fig 1). It seems that a number of stem cell populations including the BM may harbour such cells. Small numbers of side population cells derived from hepatocellular carcinoma (HCC) cell lines can propagate HCC when xenografted in the NOD/SCID mouse.²⁹ These cells may play a role in the histogenesis of HCC in cirrhosis. However, when looking specifically at BM-derived cells, no evidence was found of a contribution to HCC histogenesis in a rodent model of liver carcinogenesis induced by diethylnitrosamine and phenobarbital, although BM cell migration to the liver did occur.³⁰

THE BM CONTRIBUTES SIGNIFICANTLY TO HEPATIC NON-PARENCHYMAL CELL POPULATIONS

In contrast to hepatocytes, where derivation from the BM is limited, there is a significant contribution from BMSCs to the non-parenchymal cells within the liver. Transient cell populations such as neutrophils, lymphocytes and other inflammatory cells all traffic through the liver. They play an important role in the organ's immune regulation and, as elsewhere, are of BM haematopoietic origin. Kupffer cells are the resident monocytes, located in the hepatic sinusoids within the space of Disse. In liver disease, they have phagocytic function and release cytokines such as tumour necrosis factor- α and interleukin-6, both important mediators in hepatic inflammation and fibrogenesis. There is cumulative evidence from murine transplantation experiments that Kupffer cells are derived from the BM.^{31–33} The sinusoidal endothelium seems to have BM origins (fig 2C). Approximately 10 years ago, it was established that circulating endothelial progenitor cells (EPCs), which are of BM origin, participate in the formation of new blood vessels at ischaemic sites throughout the body.³⁴ This process is known as vasculogenesis. During liver regeneration sinusoidal endothelial cells proliferate to reconstruct hepatic sinusoids. The incorporation of BM-derived EPCs into the sinusoidal endothelium has been demonstrated in a number of animal models.^{33–35} These EPCs may have extra beneficial effects on hepatocyte regeneration, and fibrosis resolution, as will be discussed later.

Fibrogenic cells in the liver can originate from the BM

Hepatic damage during chronic liver disease is usually accompanied by progressive fibrosis. As a consequence of liver inflammation, hepatic stellate cells (HpSCs) become activated, proliferate and synthesise collagen. They display a myofibroblast phenotype histologically distinguished by expression of α -smooth muscle actin, and are thought to be central to the pathogenesis of liver fibrosis; there is, therefore, much interest in being able to clinically modify their activity. Until recently, it was thought that HpSCs were embryologically derived from neural crest tissue because they express neural cell surface markers such as glial fibrillary acidic protein and have contact with autonomic nerve endings. In a transgenic mouse model in which all neural crest cells and their progeny express yellow fluorescent protein, recent work has shown an absence of yellow fluorescent protein expression in HpSCs during development in the liver.³⁶ It has been suggested rather that HpSCs have their embryological origins in the septum transversum

mesenchyme. There is in fact a growing body of evidence to indicate that the myofibroblast population, at least in part, derives from BMSCs. In gender crossover BMT experiments using CCl₄ and thioacetamide models of liver injury, up to 70% of HpSCs and myofibroblasts associated with septal scars were BM derived.³⁷ These cells were shown to transcribe collagen, and by analysing individual sex chromosome complements, cell engraftment did not seem to occur through cell fusion. Thus, direct differentiation seemed to be the predominant mechanism. A BM contribution to hepatic fibrogenesis has also been shown in the bile duct-ligated mouse, a model of cholestatic liver disease. Hepatic collagen expression was shown using BMT from transgenic mice expressing green fluorescent protein under the control of a collagen promoter.³⁸ Interestingly, these cells were atypical for myofibroblasts, as they lacked expression of α -smooth muscle actin. The expression of CD45 suggests they may have been derived from fibrocytes, a circulating population known to deposit collagen at sites of injury.³⁹ This migratory fibrogenic cell of haematological origin is distinguished by the expression of cell surface markers such as CD34, CD45, CD11b, human leucocyte antigen-DR, and has been studied more extensively in animal models of lung and skin injury.^{40–41} The identity of its BM precursor is unclear. In contrast, tissue fibroblasts are historically considered as non-migratory and of local mesenchymal origin. Traditional boundaries between the various fibrogenic cell populations are thus becoming less distinct in the light of recent evidence. Certainly in the liver, it is likely that there is more than one population of collagen-producing cell disparate in derivation, phenotype and response to liver injury.⁴²

When human liver specimens were analysed, the same phenomenon was found. In tissue from male recipients of female liver transplants in whom liver disease had recurred, and in tissue from females with chronic liver disease who had undergone a male BMT, between 6% and 22% of hepatic scar-associated myofibroblasts were Y chromosome positive—that is, BM derived.⁴³ This has direct clinical relevance to the field of liver transplantation. Recurrence of hepatitis C, accompanied by rapid and aggressive liver fibrosis, is a major cause of graft dysfunction and failure. The implication here is that a significant proportion of the fibrotic response is in fact attributable to the recipient's cells rather than a property of the donor organ. Human BM-derived myofibroblasts have also been found in the intestine in graft versus host disease and in the lung in bronchiolitis obliterans after lung transplantation.^{44–45} Additional migration and engraftment into skin and kidney has been shown in mouse models, the location being dependent on the site of injury.^{41–46–47}

From which stem cell does the BM-derived myofibroblast arise? Here the body of data is smaller, but the suggestion is that the main protagonist is the MSC (figs 2A,B).³⁷ However, it should be noted that the aforementioned circulating fibrocyte cell may not share the same BM precursor. In effect, more than one BMSC compartment may be contributing to the scar-forming cells within the damaged liver. Likewise, different studies have collectively shown that both HSCs and MSCs may repopulate the liver or ameliorate liver disease by promoting regeneration or attenuating fibrosis. At present, the specific role of each BMSC is incompletely defined and the validity of future work is crucially dependent on exactly how donor BMSCs are isolated and characterised.

Figure 3 Potential uses of bone marrow-derived stem cells to treat liver disease and possible routes of innoculation.

WHAT REGULATES BONE MARROW STEM CELL HOMING TO THE LIVER?

Given that mounting evidence suggests that a BM–hepatic axis exists, it is important to establish what regulates the migration of BM progenitors to the liver and what factors govern their engraftment.

HSCs express the cellular receptor CXCR4, to which the natural ligand is stromal derived factor-1 (SDF-1). When the SDF-1 concentration within the BM is reduced, HSCs are recruited into the circulation and migrate along a concentration gradient.^{48–49} It has been shown that injurious stimuli such as irradiation and inflammation upregulate hepatic SDF-1 production.⁵⁰ SDF-1 expression appears to localise to the biliary epithelium, and when human CD34⁺ HSCs are introduced into the NOD/SCID mouse, cell engraftment clusters around the bile ducts. Innoculation of human SDF-1 increases homing of HSCs to the liver, and blockade of CXCR4 abrogates it. The CXCR4 receptor has also been shown on oval cells, which in vitro seem to migrate along a SDF-1 gradient. Indeed SDF-1 is significantly upregulated only in animal models of liver disease that provoke regeneration via an oval cell response.⁵¹ Interestingly

HGF, upregulated during hepatic regeneration, can augment CXCR4 expression on HSCs and potentiate SDF-1-induced migration. Stem cell factor, the production of which localises to the same area in the liver, acts synergistically with SDF-1 to induce HSC migration in vitro. HSCs express c-kit, the receptor for stem cell factor. Other factors such as matrix metalloproteinase-9 (MMP-9), which augments HSC release from the BM, and IL-8, which is upregulated in liver disease and stimulates granulocyte production of MMP-9, are also likely to be important. The literature on what determines MSC homing is more conflicting. It seems at best that only a small proportion of MSCs can express functionally active CXCR4.⁵² In in vitro migration assays, human MSCs can respond to SDF-1 and HGF gradients. MSCs express c-met, the receptor for HGF.⁵³ Using green fluorescent protein as a cell marker, MSC migration to pancreatic islets in response to SDF-1 has been demonstrated, but no in vivo experiments have investigated MSC homing to the liver.⁵⁴

Clearly, the clarification of the factors controlling BMSC migration has important implications for future treatment in liver disease. In particular, if the precise precursor of the

Table 1 Bone marrow stem cell trials in humans with liver disease published to date

Published	Patients	BM cells	Outcomes	Tissue	Ref.
<i>Stem Cells</i> 2005	Right PVE to induce contralateral segment hypertrophy before extended right partial hepatectomy Patients with liver cancer (n = 3). Controls (n = 3)	Autologous Iliac crest CD133 ⁺ 2.4–8.8 × 10 ⁶ Via portal vein	Well tolerated 2.5-fold increase in left lateral lobe compensatory hypertrophy vs control by CT volumetry criteria Followed up to surgery and discharge	None	am Esch ⁶⁸
<i>Stem Cells</i> 2006	n = 5 No controls Aetiology hepatitis B or C, alcohol, and primary sclerosing cholangitis mean age 49	Autologous Peripheral blood post G-CSF CD34 ⁺ 1 × 10 ⁶ –2 × 10 ⁸ PV-3, HA-2	Procedure and G-CSF tolerated Follow up to 60 days Some observed trends of improvement in albumin or bilirubin, most marked in primary sclerosing cholangitis patient	None	Gordon ⁶⁹
<i>Stem Cells</i> 2006	Patients with cirrhosis (n = 9) No controls Aetiology hepatitis B or C Child–Pugh score 7–10 Mean age 62 years	Autologous Iliac crest Unsorted BM Mean 5.2 × 10 ⁹ Via peripheral vein	Procedure well tolerated Follow-up for 24 weeks Significant improvement in albumin and Child–Pugh scores. Minor serum changes in pro-collagen-III-peptide and HGF	Biopsy in 3 of 9 ↑ PCNA BMSC Not labelled	Teraï ⁷⁰

HA, hepatic artery; PVE; portal vein embolisation; PCNA, proliferating cell nuclear antigen; PV, portal vein.

BM-derived myofibroblast is identified and its migration pathway elucidated, then the development of liver-specific anti-fibrotic therapies may become possible.

CAN BMSC TREATMENT BE DEVELOPED FOR THE TREATMENT OF LIVER DISEASE?

Given the progression of BMSC biology in the past few years, the question arises: can BM cells be used to treat liver disease? To address this question, we will go on to appraise some of the data available from animal models and discuss the advent of stem cell treatment in small clinical trials.

Animal data

The implication of the aforementioned “proof of principle” experiments is that some hepatocyte regeneration may be achieved through BMSC transplantation. This can correct metabolic deficiency states and can also lead to measurable improvements in hepatic function after damage. Whether engraftment and organ restitution continues in the long term has not been answered.

One pathway by which recovery can occur in chronic liver disease is through a reduction in hepatic fibrosis. When MSCs in vitro were induced to adopt a hepatocyte phenotype and then transplanted intravenously into non-irradiated CCl₄-damaged recipients, a histological decrease in hepatic fibrosis and a rise in serum albumin were noted.⁵⁵ Likewise in a similar animal model and experimental paradigm, the transplantation of a BM mononuclear MSC subpopulation (characterised in culture by the expression of fetal liver kinase-1, also found on EPCs and inducible on macrophages) led to a reduction in liver fibrosis when infused early enough after the onset of injury.⁵⁶ CCl₄-induced murine hepatic fibrosis also seemed to be ameliorated via the infusion of a Liv-8-depleted BM subfraction halfway through a CCl₄ intoxication regimen.⁵⁷ Here the cells were found in fibrous bands in the liver, colocalising with areas of dense MMP-9 expression. It may be, at least in part, that the anti-fibrotic property of BM cells is conferred by the infusion of macrophages (which express MMPs central to the degradation of collagen bands). It has been clearly shown that BM-derived macrophages

are crucial to the resolution of CCl₄-induced liver fibrosis during the recovery phase after injury.⁵⁸

Another possible explanation for the reduction in fibrosis is that migrating BM cells increase hepatocyte proliferation and suppress fibrogenesis by supplying growth factors and cytokines critical to the recovery process. Survival after massive CCl₄ injury is augmented by the transplantation of EPCs into non-irradiated mice concordant with an increase in HGF and vascular endothelial growth factor levels in the damaged livers (fig 2C).⁵⁵ Incorporation of transplanted cells into the sinusoidal endothelium was found, but no convincing hepatocyte transdifferentiation was seen. The same group has also shown amelioration of liver fibrosis with EPC treatment, in the presence of increased HGF and vascular endothelial growth factor, and a reduction in the pro-fibrotic mediator transforming growth factor-β.⁵⁹

Growth factors such as HGF are known to have a direct effect on myofibroblasts. In vitro HGF interferes with platelet-derived growth factor signalling, a potent myofibroblast mitogen, and, in rat liver injury, it reduces myofibroblast number through apoptosis.⁶⁰ Interestingly, in the bile duct-ligated model of cholestatic injury, HGF reduces the number of periportal myofibroblasts and hence diminishes fibrosis.⁶¹ If these cells are isolated and cultured in the presence of HGF, they show a reduction in transforming growth factor-β-induced activation, reportedly via an inhibition of epithelial mesenchymal transition. Epithelial mesenchymal transition is being investigated as a mechanism by which periportal myofibroblasts can be derived through direct transition from damaged biliary epithelia in cholestatic liver diseases.

The stem cell niche

Animal research on the therapeutic application of BMSCs throws up conflicting results as to the success of donor cell engraftment and organ reconstitution. In part, this may be owing to important differences in the experimental procedure followed. The stem cell niche defines the microenvironment of a stem cell within its organ, and is well described in the gut, skin and BM. In the liver, the canals of Hering are the most likely equivalent. The niche is conducive to the maintenance of a stem cell population and can influence its differentiation into

specific progenitors. Stromal cells are considered central to the regulation of the niche. The activity and fate of exogenously applied stem cells is likely to depend on whether they can integrate into their respective niches. This may depend on whether the existing stem cells within the niche have been disrupted or depleted. In the BM, myeloablation through irradiation will have this effect. In the liver, toxic damage (eg, with CCl_4) can alter the local niche. The fate of transplanted BM cells may thus be determined by whether they are introduced locally into the liver or whether their inoculation is peripheral, via the BM. The prior manipulation of the stem cell niche in the recipient is likely to be an important factor in the outcome.

Clinical trials of BM treatment in liver disease

Can preclinical research on BMSCs be translated to treat patients with liver disease (fig 3)? In cardiology, trials with BMSCs are at more advanced stages. There are now many studies in the literature with controlled, often double-blinded trials of up to 70 patients showing variable success. The majority have employed autologous BMSC intracoronary transplantation after myocardial infarction. Using outcome measures such as improvement in left ventricular function and reduction in infarction size, some studies have demonstrated clinical benefits, whereas in others the differences have been less significant.^{62–64} The application of BM cell treatment in liver disease is not as far advanced. It was shown that BMT (for haematological malignancy) from hepatitis B immune donors could eradicate chronic hepatitis B infection in human leucocyte antigen-matched recipients through the adoptive transfer of hepatitis B core antigen reactive CD4^+ T cell subsets.⁶⁵ This example describes an inadvertent use of BM cell treatment and does not specifically involve stem cells, but does demonstrate a proof of principle that BM cells can be used to treat liver disease. Turning to patients with chronic liver disease, there does not seem to be an increase in circulating BM-derived stem cells (defined as CD34^+) at times of acute decompensation.⁶⁶ Nor does there seem to be a consistent improvement in liver function when G-CSF is given to patients with cirrhosis to increase the CD34^+ cell count in peripheral blood, though isolated improvements in some biochemical indices are noted.⁶⁷ G-CSF, however, was shown to be safe and well-tolerated in cirrhotics. So can BMSC treatment be used to restore hepatic function?

There are only a handful of clinical trials, all of which are small-scale, uncontrolled feasibility studies (table 1). The first study looked at patients with liver cancer undergoing portal vein embolisation to induce contralateral lobe hypertrophy and thereby increase the size of the future remnant liver volume before an extensive partial hepatectomy.⁶⁸ Accelerated hepatic regeneration was demonstrated in three of these patients after the infusion of autologous CD133^+ BM cells. By CT criteria, the left lateral segments hypertrophied by two and a half times more than in non-BM cell-treated controls. Another preliminary uncontrolled study in five patients with cirrhosis showed a transient improvement in clinical parameters such as serum bilirubin and albumin over 60 days after portal vein or hepatic artery infusion of 1×10^6 to 2×10^8 autologous CD34^+ BMSCs. Again feasibility and safety were demonstrated.⁶⁹ The only other published clinical trial involved nine patients with

cirrhosis who received portal vein infusion of 5.2×10^9 autologous unsorted BM cells.⁷⁰ Follow-up was longer, at 24 weeks, and patients showed some improvement in Child–Pugh score and albumin. Liver biopsies, when taken, showed increases in proliferating cell nuclear antigen staining, an indirect marker of hepatocyte turnover; however, there was no control arm. Interestingly, a recent case report describes the use of autologous BMSCs as rescue treatment for hepatic failure in a 67-year-old man ineligible for liver transplantation.⁷¹ The portal venous infusion of 5×10^6 CD34^+ cells, obtained from peripheral blood after G-CSF induction, led to an apparent rapid improvement in hepatic synthetic function in this patient, who suffered from an acute drug-related hepatitis superimposed on chronic alcoholic liver disease. A liver biopsy performed 20 days after BMSC treatment was reported as showing increased hepatocyte replication around necrotic foci, although BMSCs were not identifiable as they were not labelled with markers before transplantation.

In none of the clinical trials so far has colonisation or even engraftment of transplanted cells been demonstrated in recipient livers. It is conceivable that the variable change in parameters of hepatic function may be occurring through the supply of growth factors promoting liver regeneration and fibrosis resolution. This in itself may be a sufficiently satisfactory end point. As a concept, using a patient's own BMSCs to bring about improvements in liver function seems an attractive option. Clearly, strategies other than orthotopic liver transplantation are required in the near future as the burden of chronic liver disease increases. Using hepatocytes or their precursors for cell-based treatment is beset by the fact that the availability of such cells depends on the procurement of cadaveric organs, which are in short supply. Embryological or foetal stem cell treatment is very promising but has considerable practical and ethical hurdles still to be overcome before this technology can be implemented. Using BMSCs for cell treatment is likely to have its advantages and disadvantages. An obvious advantage of BMSCs is that accessing them is easy and their supply is in theory unlimited. In addition immunosuppression may not be needed as the source of cells could be autologous. There are well-established haematological protocols for the *in vitro* expansion of BMSCs to treat other conditions, so a paucity of transplantable cells to treat liver disorders may not be a major obstacle.

There are however, we believe, still important theoretical and practical questions that need to be addressed before BMSC treatment for liver disease can become a viable clinical reality. There is very little evidence that BMSCs can make hepatocytes at a level that could be clinically useful, nor has stable or long-term engraftment been demonstrated. It is more probable that a realistic goal of BMSC treatment is to stimulate the regeneration of endogenous parenchymal cells or enhance fibrous matrix degradation. It appears that BMSC treatment can create a milieu conducive to liver regeneration through the transient supply of growth factors, but it is likely that repeated treatment would be required in clinical practice; this has not yet been studied. It is also apparent that cell fusion may be a vehicle by which the genetic status of host hepatocytes can be altered, although it is not yet clear whether the cells so-derived have replication potential over the long term. It is important to take into account the potential that stem cells may have for

malignant transformation. There is mounting evidence that the majority of cancers arise from stem cell clones. In mouse models of regeneration, cell fusion often results in polyploidy or chromosomal instability. It has become increasingly evident that the cellular origin of HCC is the oval cell or hepatic progenitor cell. Gastric carcinoma can arise from BM-derived cells in a mouse model of chronic *Helicobacter felis* infection.⁷² In addition, it has been shown that BM-derived myofibroblasts contribute to the stroma around pancreatic insulinomas in mice.⁷³ This raises a theoretical concern that BMSC treatment may accelerate carcinogenesis in patients with liver disease. There is already a well-documented incidence of HCC in patients with cirrhosis, the precise cohort for which stem cell treatment may be most needed.

What is vital is a coherent understanding of the effect of the individual components of the BMSC populations and their progeny on the diseased liver. This will facilitate the rational planning of BMSC treatment. It can be concluded that not all cells have the same effect on liver pathology. It has been noted that profibrotic cells can be of BM origin. There is surely a danger of making the situation worse for patients with cirrhosis unless the exact precursors of the different liver cell populations and the precise mechanisms that dictate their differential migration and engraftment are more fully understood. That said, there is of course the theoretical potential to exploit the BM–hepatic fibrogenic axis to influence and deliver anti-fibrotic treatments through the BM. This is an area in which future investigation may prove rewarding.

SUMMARY

BMSCs can interact with disease processes in a number of organs including the liver. Under most circumstances, current evidence suggests that BMSCs do not play a large role in the repopulation of the hepatic parenchyma, and cell fusion seems to be the predominant process when this does occur. BMSCs may support liver repair, however, through the delivery of growth factors that promote liver regeneration, fibrosis resolution or new blood vessel formation. Conversely, subsets of BMSCs can also contribute to fibrogenesis within the liver in response to injury. The evidence to inform which BMSCs are involved is conflicting, reflecting variances in how individual stem cells are defined and highlighting difficulties in demarcating clear boundaries between the different compartments. An understanding of what regulates BMSC homing to the liver is emerging, and SDF-1/CXCR4 signalling seems central to this. Trials of BMSC treatment in patients with liver disease have already started but are still at a very preliminary stage. A more comprehensive understanding of BMSC physiology in animal models of liver disease is essential to improve the likelihood that such treatment will result in successful clinical treatments in the future.

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Authors' affiliations

Y N Kallis, Department of Medicine, St Mary's Hospital Campus, Imperial College, London, UK

M R Alison, Institute of Cell and Molecular Science, Queen Mary School of Medicine and Dentistry, London, UK

S J Forbes, Tissue Fibrosis and Remodelling Laboratory, MRC/University of Edinburgh Centre for Inflammation Research, Edinburgh, UK

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