

Stem Cells as a Treatment for Chronic Liver Disease and Diabetes

N. Levičar¹ (✉) · I. Dimarakis¹ · C. Flores² · J. Tracey¹ · M. Y. Gordon² · N. A. Habib¹

¹Department of Surgical Oncology and Technology, Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK
n.levicar@imperial.ac.uk

²Department of Haematology, Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

1	Cell Therapy	244
2	Haematopoietic Stem Cells	244
3	Injured Liver	245
3.1	Generation of Hepatocytes by Haematopoietic Stem Cells	246
3.2	Haematopoietic Stem Cell Therapy for Liver Diseases	247
3.2.1	Animal Studies	247
3.2.2	Human Studies	249
4	Diabetes	250
4.1	Haematopoietic Stem Cells for β -Cell Generation	251
4.2	Haematopoietic Stem Cells for Diabetes	254
4.2.1	Animal Studies	254
4.2.2	Human Studies	256
5	Conclusions	257
	References	257

Abstract Advances in stem cell biology and the discovery of pluripotent stem cells have made the prospect of cell therapy and tissue regeneration a clinical reality. Cell therapies hold great promise to repair, restore, replace or regenerate affected organs and may perform better than any pharmacological or mechanical device. There is an accumulating body of evidence supporting the contribution of adult stem cells, in particular those of bone marrow origin, to liver and pancreatic islet cell regeneration. In this review, we will focus on the cell therapy for the diseased liver and pancreas by adult haematopoietic stem cells, as well as their possible contribution and application to tissue regeneration. Furthermore, recent progress in the generation, culture and targeted differentiation of human haematopoietic stem cells to hepatic and pancreatic lineages will be discussed. We will also explore the possibility that stem cell technology may lead to the development of clinical modalities for human liver disease and diabetes.

Keywords Stem cell therapy · Haematopoietic stem cells · Liver disease · Diabetes

1 Cell Therapy

Cell therapy has emerged as a novel approach for the treatment of many human degenerative diseases. Cell-based regenerative strategies aim to replace, repair or enhance the biological function of damaged tissues or organs by utilising cells and bioactive molecules to trigger, enhance, support and complement the residual capacity for repair. This can be achieved by the transplantation of cells, which are typically manipulated *ex vivo*, into a target organ in sufficient numbers for them to survive long enough to restore the normal function of organs and tissues. Possible candidate cells to be used include autologous primary cells, cell lines, various stem cells including bone marrow stem cells, cord blood stem cells and embryonic stem (ES) cells (Fodor 2003).

In recent years, advances in stem cell biology, including embryonic and somatic stem cells, have made the prospect of tissue regeneration a potential clinical reality, and several studies have shown the great promise that stem cells hold for therapy (Assmus et al. 2002; Wollert et al. 2004). Despite the unquestioned totipotency of ES cells, there are numerous unanswered biological questions as to the regulation of their growth and differentiation. The safety profile of unselected ES cells for transplantation early on demonstrated dysregulated cell growth resulting in teratoma formation (Reubinoff et al. 2000). Moreover, the ethical and legal issues associated with ES cells have shifted the focus to adult stem cells, and their regenerative potential has been under intense investigation.

The main role of adult stem cells, which are present in approximately 1%–2% of the total cell population within a specific tissue, is to replenish of the tissue's functional cells in appropriate proportions and numbers in response to 'wear and tear' loss or direct organ damage (Fang et al. 2004). They are vital in the maintenance of tissue homeostasis by continuously contributing to tissue regeneration and replacing cell lost during apoptosis or direct injury (Li and Xie 2005). Adult stem and progenitor cells possess the capacity of self-renewal and differentiation into one or more mature cell types. They are able to maintain their populations within the human body through asymmetric and symmetric divisions, resulting in the creation of differentiated and undifferentiated progeny (Preston et al. 2003). These properties make them ideal candidates for stem cell-based therapies and tissue engineering.

2 Haematopoietic Stem Cells

Haematopoietic stem cells (HSC) are multipotent bone marrow cells that sustain the formation of the blood and immune system throughout life. First identified in 1961, HSC have been by far the best characterised and most stud-

ied example of adult stem cells (Till and McCulloch 1961). The bone marrow compartment is largely made up of committed progenitor cells, non-circulating stromal cells [called mesenchymal stem cells (MSC)] that have the ability to develop into mesenchymal lineages and HSC (Pittenger et al. 1999; Bianco et al. 2001). It was previously thought that adult stem cells were lineage restricted, but recent studies demonstrated that bone marrow-derived progenitors in addition to haematopoiesis also participate in regeneration of ischaemic myocardium (Orlic et al. 2001), damaged skeletal muscle (Gussoni et al. 1999) and neurogenesis (Mezey et al. 2000).

MSC are the second major population of stem cells residing in bone marrow. MSC can be isolated as a growing adherent cell population and can differentiate into osteoblasts, adipocytes and chondrocytes (Pittenger et al. 1999).

3

Injured Liver

Liver diseases impose a heavy burden on society and affect approximately 17% of the population. Cirrhosis, the end result of long-term liver damage, has long been an important cause of death in UK and showed large rises in death rates over the past 20 years (Ministry of Health 2001). The main causes of cirrhosis globally are hepatitis B and C and alcohol abuse. Changing patterns of alcohol consumption and the increasing incidence of obesity and diabetes suggest that the burden of fibrosis and cirrhosis related to alcohol and non-alcohol steatohepatitis (NASH) will continue to increase (Fallowfield and Iredale 2004).

Cirrhosis is a progressive liver disease and is marked by the gradual destruction of liver tissue over time. Persistent injuries lead to hepatic scarring (fibrosis), which, if unopposed, leads to cirrhosis and demise of liver function. End-stage liver fibrosis is cirrhosis, whereby normal liver architecture is disrupted by fibrotic bands, parenchymal nodules and vascular distortion. Portal hypertension and hepatocyte dysfunction are the end results and give rise to major systemic complications and premature death. At the cirrhotic stage, liver disease is considered irreversible and the only solution is orthotopic liver transplantation (OLT). However, the increasing shortage of donor organs restricts liver transplantation. With the widening donor-recipient gap, the increasing incidence of liver disease, life-long dependence on immunosuppression and the poor outcome in patients not supported by liver transplantation, there is obviously a demand for new strategies to supplement OLT.

According to current concepts of liver regeneration, the liver has three levels of cells that can respond to liver injury and loss of hepatocytes (Sell 2001). First, mature hepatocytes, which are numerous, respond to mild liver injury by 1 to 2 cell cycles. Second, intra-organ ductal progenitor cells, which are less numerous, respond by longer and limited proliferation. Third, stem cells

entering from the circulation participate in liver regeneration. These cells, in part of bone marrow origin, enter the liver in a seemingly random distribution, as isolated cells, or in a portal and periportal distribution when there is marked injury (Petersen et al. 1999). In this latter mode, responding to severe injury, they enter first as an intermediate cell population, which then mature into hepatocytes.

New strategies for generating a viable source of healthy hepatocytes for treating hepatic disorders are under investigation. Potential sources include the expansion of existing hepatocytes, ES cells, progenitor/stem cells in the liver, and bone marrow stem cells. Direct transplantation of hepatocytes has already been successfully tried in a small number of patients as a bridge to OLT or as a therapeutic alternative but there remain several limitations, which mainly include limited hepatocyte amplification, the fact that replacement of less than 10% of the liver mass is achieved, the short duration of the functioning replacement cells and the need for immunosuppression (Ohashi et al. 2001; Najimi and Sokal 2005). Additionally, the number of patients that can be treated in this manner is severely limited due to the paucity of healthy donors. This limited success fuelled the interest in haematopoietic stem cells for hepatic disorders.

3.1

Generation of Hepatocytes by Haematopoietic Stem Cells

In vitro studies have demonstrated the potential of bone marrow stem cells to differentiate towards the hepatic lineage. Oh et al. (2000) and Miyazaki et al. (2002) have shown that rat bone marrow contains a subpopulation (3%) of cells co-expressing haematopoietic stem cell markers (CD34, c-kit, Thy-1), α -fetoprotein (AFP) and c-met. They have also demonstrated the expression of albumin, a marker of terminally differentiated hepatocytes, after culturing crude bone marrow in the presence of hepatocyte growth factor (HGF) and epidermal growth factor (EGF). Similar observations were made by Okumoto et al. (2003), where rat bone marrow cells enriched for Sca-1 began expressing liver-enriched transcription factor HNF1 α and cytokeratin 8 (CK8) after being cultured in the presence of HGF. Moreover, Ratajczak et al. (2004) demonstrated that a subpopulation of murine mononuclear bone marrow cells isolated by chemotaxis in response to the α -chemokine stromal-derived factor-1 (SDF-1) expressed messenger RNA (mRNA) for AFP and a population enriched for Sca-1 expressed mRNA for AFP, c-met and CK19. Purified murine HSC were able to differentiate into liver-like cells expressing (AFP, GATA4, HNF4, HNF3 β , HNF1 α) and mature hepatocyte markers (CK18, albumin, transferrin) when co-cultured with injured liver tissue, suggesting that micro-environmental cues are responsible for conversion (Jang et al. 2004).

Studies on human bone marrow confirmed observations from rodent models and showed that human bone marrow cells have the potential to differentiate into liver-like cells. When cultured on collagen matrix and in the presence of

HGF they expressed liver-specific genes, albumin and CK19 (Fiegel et al. 2003). Furthermore, when selected by SDF-1 chemotaxis they appear to be multipotent and express AFP (Kucia et al. 2004). Numerous cytokines and growth factors have been shown to have potent effects on hepatic growth and differentiation under *in vitro* culture conditions (Heng et al. 2005). HGF, EGF, transforming growth factor (TGF), and acid fibroblast growth factor (aFGF) are the most commonly used in the majority of studies and have been shown to promote hepatic differentiation *in vitro* (Block et al. 1996; Michalopoulos et al. 2003).

Like HSC, a subpopulation of human MSC known as multipotent adult progenitor cells (MAPC) has the potential to differentiate into hepatocyte-like cells in the presence of HGF and fibroblast growth factor-4 (FGF-4) (Schwartz et al. 2002). However, the MAPC culture is fastidious, with a substantial delay between the isolation and the appearance of hepatocyte-like cells, which calls into question their use in the clinic. *In vitro* hepatic differentiation of MSC in the presence of HGF and oncostatin M was confirmed by another group (Lee et al. 2004). Again, the differentiation process was lengthy but they demonstrated the expression of liver-specific genes in differentiated cells and other characteristics of liver cells, including albumin production, glycogen storage, urea secretion, uptake of low-density lipoprotein, and phenobarbital-inducible cytochrome P450 activity.

3.2

Haematopoietic Stem Cell Therapy for Liver Diseases

3.2.1

Animal Studies

Liver has long been known to exhibit considerable regenerative potential, but it has been only recently that we began to understand the implication of stem/progenitor cells in this process. The extrahepatic stem cells such as HSC are of particular interest since they are easily accessible.

Petersen et al. (1999) were first to show that bone marrow stem cells could be a potential source of hepatic oval cells. The liver injury was induced with 2-AAF-CCl₄, followed by cross-sex bone marrow transplantation. Regenerated hepatic cells were shown to be of bone marrow origin, demonstrated by using markers for the Y chromosome, dipeptidyl peptidase IV enzyme, and L21-6 antigen to identify donor-derived cells.

The other demonstration of hepatocyte regeneration from bone marrow cells is the extensive repopulation of damaged livers of fumarylacetoacetate hydrolase (FAH) mice, an inducible animal model of tyrosinemia type 1 (Grompe et al. 1995), a lethal hereditary liver disease (Lagasse et al. 2000). Intravenous injection of adult bone marrow cells in the FAH^(-/-) mouse rescued the mouse and restored the biochemical function of its liver. The liver repopulation by bone marrow cells was slow, although significant. The first hepatocytes of

bone marrow origin appeared 7 weeks after transplantation. However, later on, at 22 weeks, one-third of the liver comprised bone marrow-derived cells. This suggested that bone marrow stem cells contribute to hepatocyte generation in the presence of injury, where the regenerative potential of hepatocytes is impaired. Furthermore, recent data have demonstrated that bone marrow stem cells injected during liver injury can reduce the resulting liver fibrosis (Sakaida et al. 2004). The exact mechanism of this therapeutic effect is not fully understood yet, but it may be facilitated by the matrix metalloproteases, which enable degradation of hepatic scars and are expressed by bone marrow stem cells. More studies have shown that HSC engraft, repopulate and have survival advantage when transplanted into injured liver. Mallet et al. (2002) used JO₂ antibody, the murine anti-Fas agonist, to induce hepatic apoptosis. Unfractionated bone marrow cells expressing Bcl-2 under the control of a liver-specific promoter were transplanted into normal mice. Some mice received repeated weekly injections of JO₂ antibody to induce liver injury. Bone marrow-derived hepatocytes expressing Bcl-2 were only seen in the liver of the mice, which received JO₂ antibody injections. Moreover, in mice with induced liver cirrhosis, 25% of the recipient liver was repopulated in 4 weeks by bone marrow-derived hepatocytes (Terai et al. 2003). Jang et al. (2004) reported that murine HSC converted into viable hepatocytes with increasing liver injury. They noticed that liver function was restored 2–7 days after transplantation of murine Fr25lin⁻ cells into irradiated and CCl₄ injured murine liver, suggesting that HSC contribute to the regeneration of injured liver by differentiating into functional hepatocytes. Misawa et al. (2006) used desialylated bone marrow cells in order to increase their accumulation in rat livers in a rodent model of human hepatic Wilson's disease. They demonstrated that direct accumulation of desialylated bone marrow cells into liver increased the proportion of functional bone marrow cell-derived hepatocytes. However, some studies have shown that bone marrow stem cells can repopulate liver even in the absence of liver injury. In one study, Theise et al. (2000a) transplanted unfractionated male bone marrow or CD34⁺lin⁻ cells into irradiated female mice and looked for bone marrow-derived hepatocytes. They identified up to 2.2% Y-positive hepatocytes at 7 days and 2 months or longer post-transplantation. Moreover, positive fluorescent in situ hybridization (FISH) for the Y-chromosome and albumin mRNA confirmed male-derived cells were mature hepatocytes, suggesting that hepatocytes can be derived from bone marrow cells in the absence of severe acute injury. Krause et al. (2001) injected single male HSC into irradiated mice and obtained engraftment in several organs, including liver, gastrointestinal tract, bronchus and skin of recipient animals. In support of the findings, Wang et al. (2003) found albumin-expressing hepatocyte-like cells in the livers of mice transplanted with highly purified HSC.

In contrast, several other studies failed to show trans-differentiation of bone marrow stem cells into liver parenchyma. Wagers et al. (2002) transplanted single HSC into lethally irradiated mice and showed that HSC reconstituted blood

leukocytes of irradiated mice, but did not contribute to non-haematopoietic tissues, including liver, brain, kidney, gut, and muscle. Kanazawa and Verma (2003) used various liver injury models to assess hepatic regeneration by bone marrow stem cells. By using three different models—CCl₄ treatment, albumin-urokinase transgenic mouse [TgN(Alb1Plau)], or hepatitis B transgenic mouse [TgN(Alb1HBV)]—they concluded there was little or no contribution of bone marrow cells to the replacement of injured livers. Similarly, Dahlke et al. (2003) concluded that bone marrow cell infusion was not able to enhance liver regeneration in a retrorsine/CCl₄ model of acute liver injury. It was shown that there is only a small contribution of bone marrow stem cell to liver regeneration after chronic liver injury. Vig et al. (2006) have found only 4.7% bone marrow-derived hepatocytes at 3 months after transplantation and even less (1.6%) at 6 months after bone marrow transplantation.

Although views concerning the contribution of HSC to hepatocyte lineages *in vivo* still remain divided, the differences between the studies may in part reflect the types of cells used, different injury models used and the method used to detect engrafted stem cells.

3.2.2

Human Studies

Several studies have also shown the presence of cells of bone marrow origin in the human liver. Alison et al. (2000) showed that adult human liver cells can be derived from the stem cells originated in bone marrow. They examined livers derived from female patients who received bone marrow transplantation from a male donor and have found Y-chromosome and CK8-positive hepatocytes, suggesting that extrahepatic stem cells can colonise the liver. Similarly, Theise et al. (2000b) investigated archival autopsy and biopsy liver specimens from recipients of sex-mismatched therapeutic bone marrow transplantation and orthotopic liver transplantations. They identified hepatocytes and cholangiocytes of bone marrow origin by immunocytochemistry staining for CK8, CK18 and CK19 and FISH analysis for the Y-chromosome. Using double staining analysis, they found a large number of engrafted hepatocytes (4%–43%) and cholangiocytes (4%–38%), showing that they can be derived and differentiated from bone marrow and replenish hepatic parenchymal cells. Although Korbling et al. (2002) confirmed bone marrow-derived hepatocytes in liver biopsies of sex-mismatched bone marrow transplantation, they found less significant numbers (4%–7%) than Theise et al. (2000b). Similarly, Ng et al. (2003) showed that most of the recipient-derived cells in the liver allografts were macrophages/Kupffer cells and only a small proportion of hepatocytes (1.6%) was recipient derived. Two other studies of liver transplant patients did not detect bone marrow-derived hepatocytes at all (Fogt et al. 2002; Wu et al. 2003).

The difference and inconsistent results of the published studies could be due to use of different techniques to identify recipient-derived hepatocytes in

transplanted patients. Also, various markers can be used for hepatocyte identification, and the accuracy of the methods used for identification is variable.

Although studies have shown that bone marrow stem cells could give rise to hepatocytes, the use of bone marrow stem cells as therapeutic agents is still in its infancy. So far, only three studies have published the results from the clinical use of bone marrow stem cells for liver insufficiency. The first clinical study was performed by am Esch et al. (2005) who infused 3 patients with autologous CD133⁺ cells subsequent to portal vein embolisation of right liver segments. Computerised tomography scan volumetry showed a 2.5-fold increase in the growth rate of the left lateral segments compared to the control group of 3 patients that had been subjected to portal vein embolisation without bone marrow stem cell administration. Despite the small number of patients and the lack of an adequately sized randomised control group, these data suggested that cell therapy enhances and accelerates liver regeneration. The second clinical study using adult haematopoietic stem cells in treatment of liver diseases was carried out by our group (Gordon et al. 2006). We have performed a phase I clinical trial in which five patients with liver insufficiency were given granulocyte colony-stimulating factor (G-CSF) to mobilise their stem cells for collection by leukapheresis. Between 1×10^6 and 2×10^8 CD34⁺ cells were injected into either the portal vein or hepatic artery. Of the 5 patients, 3 showed improvement in serum bilirubin and 4 in serum albumin. Clinically, the procedure was well tolerated with no observed procedure-related complications. The data clearly suggested the contribution of stem cells to the regeneration of liver damage and are encouraging for the future development of stem cell therapy for liver diseases. A more recent study confirmed the observations from the previous two studies. Terai et al. (2006) treated nine liver cirrhosis patients with autologous bone marrow. They infused $5.20 \pm 0.63 \times 10^9$ mononuclear cells (CD34⁺, CD45⁺, c-kit⁺) via the peripheral vein and followed the patients for 24 weeks. Significant improvements in serum albumin levels, total protein and improved Child-Pugh scores were observed at 24 weeks after therapy, suggesting that bone marrow cell therapy should be considered as a novel treatment for liver cirrhosis patients.

4

Diabetes

Type 1 diabetes accounts for only 5%–10% of all diabetes cases worldwide but its incidence is increasing with current estimates of those affected at approximately 10^5 in the UK and 10^6 in the USA (Burns et al. 2004; Daneman 2006). Type 1 (insulin-dependent) diabetes is a chronic disease affecting genetically predisposed individuals in which insulin-secreting β -cells within pancreatic islets of Langerhans are selectively and irreversibly destroyed by autoimmune assault. Once activated the continued destruction of β -cells leads

to a progressive loss of insulin, then to clinical diabetes, and finally—in almost all affected—to a state of absolute insulin deficiency (Devendra et al. 2004). Type 2 diabetes was once considered a disease of wealthy nations but now it is a global affliction. The incidence of type 2 (adult onset or insulin resistant) diabetes is increasing globally and has reached pandemic proportions (Petersen and Shulman 2006). Estimates by the International Diabetes Federation (IDF) anticipate that the worldwide incidence of diabetes among those 20–79 years old will increase by around 70% in the next 20 years (International Diabetes Federation 2006). Although the aetiology of type 2 diabetes remains obscure, obesity and a sedentary lifestyle are the most common epidemiologic factors associated with development of the disease.

The common denominator in both types 1 and 2 diabetes is a decrease in β -cell mass resulting in an absolute or relative state of insulin insufficiency, respectively. Long-term normalisation of glucose metabolism is a prerequisite for prevention of secondary complications and to date has only been achieved with transplantation of the whole organ or with a reasonable number of islets. Recent success with the Edmonton protocol in 2000 (hepatic portal vein infusion of organ donor islets of Langerhans) provided proof that the restoration of a sufficient functional β -cell mass re-establishes euglycaemia (Ryan et al. 2002). However, the chief limitation to transplantation, either whole gland or islets, is the paucity of donors. Current protocols for islet transplantation require up to 1×10^6 primary human islets per recipient the approximate equivalent of $2\text{--}4 \times 10^6$ β -cells (Burns et al. 2004). Thus, such treatment can be offered to only an estimated 0.5% of needy recipients (Lechner and Habener 2003). Additionally, differentiated β -cells cannot be expanded efficiently *in vitro* and senesce rapidly (Halvorsen et al. 2000).

Multiple approaches are now being investigated to generate insulin-producing cells *in vitro* either by genetic engineering of β -cells or by utilising various β -cell precursor cells and stem/progenitor cells with the ability to grow *in vitro* and to differentiate into insulin producing cells and ultimately into β -cells (Hess et al. 2003). ES cell usage has been the subject of both ethical and scientific debate (Frankel 2000). The demonstration of plasticity of haematopoietic stem cells has been published and has led to their study in the treatment of diabetes (Hess et al. 2003). Insulin secreting cells (β -cells or otherwise) could be transplanted into patients to help maintain blood glucose homeostasis, reduce the burden of diabetes-related complications, overcome the limitation of donor organs and provide benefit to millions of diabetics.

4.1

Haematopoietic Stem Cells for β -Cell Generation

In diabetics, 90% of insulin-secreting β -cells of the pancreatic islets of Langerhans are destroyed. Thus, the replacement of insulin-secreting β -cells would be an ideal cure. Studies of the growth, development and differentiation of pan-

cretic insulin-secreting cells have explored both embryonic and adult-derived stem cells as candidates for β -cell differentiation. Little is known about the cellular and molecular mechanisms of β -cell differentiation, but recent work has begun to pose speculative hypotheses on pancreatic cell development.

Several investigators have isolated multipotent bone marrow-derived cell populations which have been described as capable of trans-differentiating and expressing a pancreatic β -cell phenotype under differentiation culture conditions. Such populations include marrow-isolated adult multilineage-inducible (MIAMI) cells (D'Ippolito et al. 2004) and bone marrow-derived stem (BMDS) cells (Lee and Stoffel 2003; Tang et al. 2004), as well as a subpopulation of peripheral blood cells of monocytic origin which have been induced to express characteristics of pancreatic cells (Ruhnke et al. 2005). In addition, a subpopulation of multipotent human mesenchymal stem cells (hMSC) has been identified which constitutively expresses pancreatic islet transcription factors (Moriscot et al. 2005). Below, we look at these cell populations derived from the adult bone marrow, which have been successfully induced *in vitro* to genetically and phenotypically resemble pancreatic islet β -cells.

MIAMI cells, derived from whole bone marrow after 14 days in culture, are described as a homogeneous population expressing a unique set of markers. Interestingly, these cells are negative for the haematopoietic stem cell marker CD34, but express the hallmark embryonic markers Oct-4 and Rex-1, as well as markers for cells of mesodermal, ectodermal and endodermal lineages. With manipulation of culture conditions, MIAMI cells are described as being capable of undergoing neural, osteogenic, chondrogenic, adipogenic and endodermal differentiation. To promote the expression of pancreatic islet genes, D'Ippolito et al. (2004) treated MIAMI cells under conditions previously known to promote stem cell commitment to a pancreatic lineage which includes high glucose culture medium supplemented with basic fibroblast growth factor (bFGF), followed by exposure to butylated hydroxyanisole (BHA) and exendin-4. Nicotinamide, exendin-4 and activin-A were then added as factors known to induce pancreatic islet differentiation. RT-PCR analysis detected the expression of the β -cell transcription factors Beta2/NeuroD, Nkx6.1 and Isl1 in differentiation-induced cells as compared to untreated MIAMI cells. This suggests that MIAMI cells may have the potential for pancreatic β -cell differentiation and potential use *in vivo*.

As with MIAMI cells, manipulation of BMDS cell culture medium with high glucose, followed by treatments with nicotinamide and exendin, has been shown to induce cells into expressing features of pancreatic islet cells (Tang et al. 2004). Genetic analysis showed the upregulation of pancreatic β -cell genes such as *insulin I* and *II*, *Glut-2*, *nestin*, *Pdx-1*, *glucose kinase* and *Pax-6* in differentiation-treated cells. Immunocytochemical analysis also showed insulin and c-peptide synthesis in up to 20% of differentiation-treated cells. Induced cells also responded to glucose challenge and secreted insulin according to glucose concentration. These data imply that BMDS cells are

capable of differentiating into pancreatic islet cells as evidenced by genetic and phenotypic expression, as well as insulin-secreting function.

Monocytes derived from peripheral blood, known as programmable cells of monocytic origin, have also been shown to express characteristics of pancreatic β -cells in the presence of islet cell-conditioned medium, which contains EGF, HGF and nicotinamide (Ruhnke et al. 2005). After exposure to islet cell-conditioned medium, these cells expressed transcription factors involved in β -cell differentiation such as Ngn3, Nkx6.1 and NeuroD, as well as transcription factors involved in the regulation of the insulin gene. In terms of functionality, monocytic cells which were differentiated into pancreatic neo-islet cells were responsive to glucose challenge, as determined by insulin release pre- and post-differentiation induction. Programmable cells of monocytic origin were found to be genetically and functionally similar to pancreatic β -cells *in vitro*. However, *in vivo* experiments have yet to validate cell engraftment and insulin production in a diabetic model.

Moriscot et al. (2005) have identified plastic adherent hMSCs which have an Oct-4⁺ pluripotent phenotype and which constitutively express Nkx6.1, an islet-associated transcription factor. hMSCs were plated with adenoviruses encoding Ipfl, HlxB9 or FoxA2, and were then cultured alone, cultured with islet-conditioned medium or co-cultured with human islets. Various culture conditions revealed that infection with adenovirus expressing HlxB9 and FoxA2, and co-culture with human islets led to insulin secretion as well as upregulation of NeuroD and insulin I. Although the described combination of adenoviral infection and co-culture led to insulin secretion, not all transcription factors described in β -cell development were upregulated, suggesting that either these hMSCs have not terminally differentiated into pancreatic cells, or that culture conditions need to be further optimised for proper β -cell differentiation. hMSCs have the capacity of at least partial differentiation into β -cells and may be a potential target for therapeutic purposes, as they are easily isolated from adults.

In vitro work proves that adult BMDS cells are capable of differentiation into pancreatic islet β -cells and allows for manipulation cell growth and development. Several investigators have been able to induce bone marrow-derived cells into cells which phenotypically express pancreatic β -cell characteristics and respond to glucose challenge. However, cells manufactured *in vitro* have not yet demonstrated insulin secretion to the same degree as endogenous β -cells. Insulin-secreting cells generated *in vitro* have been found to secrete only about 1% of the level insulin produced by endogenous β -cells (Bonner-Weir and Weir 2005). This information supports the notion that *in vitro* cell culture may not adequately mimic the physiological microenvironment where endogenous cells develop and regenerate in the body. Importantly, assessing the engraftment and functionality of cultured cells *in vivo* is an important part of the follow-up to *ex vivo* studies.

4.2

Haematopoietic Stem Cells for Diabetes

4.2.1

Animal Studies

Promising data regarding the potential of BMDS cells to reconstitute the β -cell endocrine portion of the pancreas have been produced by Ianus and co-workers (2003). Irradiated female wild-type mice were injected with BMDS cells expressing enhanced green fluorescent protein (EGFP) via a Cre-LoxP system controlled by the active transcription of the murine insulin gene. All donors were male mice sharing the same C57BL/6 background with recipient animals. At 4–6 weeks post-transplantation, pancreatic islet tissue was harvested and EGFP-positive cells were isolated by means of fluorescence-activated cell-sorting (FACS) or manual picking under a fluorescence microscope for further analysis. Up to 3% of total cells per islet were found to express EGFP in transplanted animals with no evident expression observed in peripheral blood and bone marrow samples. RT-PCR analysis of sorted cells revealed besides insulin I and insulin II, the expression of other β -cell markers including GLUT2, IPF-1, HNF1 α , HNF1 β , HNF3 β and Pax-6. At the same time, cells lacked the common haematopoietic/leukocyte marker CD45. Finally, demonstration of glucose-dependent and incretin-enhanced insulin secretion was reported as proof of functionality.

Using the same experimental strategy as Ianus et al. (2003), another group was unable to confirm their findings (Taneera et al. 2006). Whole BMDS cells from transgenic mice in which green fluorescent protein (GFP) expression was under the control of the murine insulin promoter failed to demonstrate any signs of GFP expression within the pancreatic parenchyma of transplanted animals. As this was not informative per se of pancreatic engraftment, another series of experiments was conducted using BMDS cells expressing GFP under the control β -actin promoter. Despite the large degree of engraftment, none of the GFP-positive cells co-expressed insulin or the β -cell transcription factors Pdx-1 or Nkx6.1, while more than 99.9% expressed the pan-haematopoietic marker CD45 as well as myeloid antigens. The authors concluded that although BMDS cells demonstrated efficient pancreatic engraftment, a haematopoietic cell fate was almost exclusively adopted.

In order to study the regenerative potential of BMDS cells in diabetes mellitus, Hess et al. (2003) utilised a murine model of streptozotocin-induced pancreatic damage leading to hyperglycaemia. Hyperglycaemic mice were injected intravenously with BMDS cells from GFP transgenic donor mice. Irradiation status and c-kit expression served as intergroup comparison criteria. One of the most interesting observations made was that pancreatic injury was a prerequisite for BMDS cells taking on an insulin-producing phenotype within the pancreatic parenchyma, an observation also made by other researchers

(Mathews et al. 2004). No improvement was witnessed in the c-kit⁻ transplanted subgroup in comparison to the c-kit⁺ group or the subgroup using whole bone marrow, which points to the c-kit⁺ stem cell population as the potent subpopulation. The lack of any clinical improvement in the subgroup transplanted with irradiated whole bone marrow revealed the unlikelihood of paracrine factors being responsible for any observed improvement in the non-irradiated groups. Even though 2.5% of insulin-positive cells from islets of streptozotocin-treated animals were positive for GFP, there was no expression of Pdx-1 in these cells. In addition to their low frequency, these cells were noticed to be absent during the onset of hyperglycaemic reduction. Finally, a large proportion of donor cells documented in ductal or islet regions were of endothelial lineage, thus associating the regenerative process with various endothelial interactions. Lechner et al. (2004) transplanted BMDS cells from sex-mismatched GFP-transgenic mice into animals treated with streptozotocin in one experiment and animals that had undergone partial pancreatectomy in another. Both GFP immunostaining and Y chromosome FISH failed to detect significant evidence of donor BMDS cell trans-differentiation into β -cells.

Murine BMDS cells have been shown to undergo differentiation *in vitro* into cells that express molecular markers of pancreatic β -cells and stain positive for c-peptide and insulin (Tang et al. 2004). *In vivo* transplantation of these cells into the left renal capsule and the distal tip of the spleen of streptozotocin-mediated diabetic mice not only reversed the existing hyperglycaemia, but also restored the animals' ability to respond to *in vivo* glucose challenges. Another interesting approach is that of multiple in contrast to single injections of BMDS cells in order to restore normoglycaemia (Banerjee et al. 2005). Banerjee et al. (2005) demonstrated improved glycaemic control in addition to histopathologic evidence of pancreatic islet regeneration following multiple intravenous injections of BMDS cells in streptozotocin-treated mice. The fact that BMDS cells were harvested from experimental-diabetic mice is evidence of the retained regenerative potential of the bone marrow.

It is quite striking that a number of groups have not been able to reproduce the results of Ianus et al. (2003). Most authors noted a number of technical considerations in an attempt to explain this. Many groups have reversed the process of identifying pancreatic-specific markers in engrafted cells by trying to identify cellular populations expressing such markers prior to any *in vitro* manipulation. Human mononuclear umbilical cord blood cells have been reported to express a vast number of cell markers and transcription factors necessary for β -cell differentiation (Pessina et al. 2004). When transplanted into non-obese diabetic mice with autoimmune type 1 diabetes, human umbilical cord blood mononuclear cells were able to reduce blood glucose levels and improve survival compared to untreated animals (Ende et al. 2004). The argument regarding the true nature and fate of candidate 'insulin-producing' cells remains unresolved (Sipione et al. 2004), as they may not be true β -cell precursors and furthermore may be unsuitable for clinical transplantation.

Pre-transplantation *in vitro* manipulation (Oh et al. 2004; Tang et al. 2004) may also provide a useful tool for delivering large numbers of predefined cells, thus avoiding complications such as suboptimal delivery rates and cell differentiation down non-pancreatic pathways.

Recent data have also documented the capacity for self-renewal of post-natal murine β -cells (Dor et al. 2004), thus casting a shadow of uncertainty over scientists' expectation of multipotent stem cells. The existence of unknown molecular and cellular pathways is highly likely. Endothelial signals have been linked with induction of pancreatic differentiation (Lammert et al. 2001), an association that may underlie observations in some of the above studies (Choi et al. 2003; Hess et al. 2003; Mathews et al. 2004). Many studies have failed to demonstrate expression of insulin or Pdx-1 in donor bone marrow-derived cells seen in peri-ductal or peri-islet locations (Choi et al. 2003; Hess et al. 2003; Mathews et al. 2004; Taneera et al. 2006), whilst many of these cells express endothelial markers (Choi et al. 2003; Hess et al. 2003; Mathews et al. 2004). Further to identifying actual potent subpopulations (Hess et al. 2003), a few technical considerations may come in useful. A dose-dependent effect has been noted (Ende et al. 2004), suggesting possible population expansion to be required prior to clinical transplantation. Finally, the demonstration of multi-step cell delivery being more efficient long-term (Banerjee et al. 2005) also underlines the possibility of introducing this principle in experimental and possibly clinical work.

4.2.2

Human Studies

The first reported data on cellular therapy for type 1 and 2 diabetes were presented by Fernandez Vina et al. (2006a, b). They have treated 23 patients with type 1 diabetes with $CD34^+CD38^-$ cells isolated from bone marrow and followed them up for 90 days. After 90 days from cell transplantation, the blood sugar decreased by 9.7% and c-peptide significantly increased by 55%. It was also observed that the exogenous insulin, taken daily by the patients, decreased by 17% suggesting that autologous bone marrow stem cells could improve pancreatic function. Similar results were obtained for type 2 diabetes patients where autologous $CD34^+CD38^-$ cells were transplanted via spleen artery into 16 patients. The blood sugar significantly decreased by 27% 90-days post-transplantation, while c-peptide and insulin increased by 26% and 19%, respectively. Even more impressive is the fact that 90 days post-transplantation, 84% of treated patients did not need any more anti-diabetic drugs or insulin.

5 Conclusions

Over the past 5 years attention has been focussed sharply on stem cells and the extraordinary potential that they offer in treating a number of currently intractable human diseases. However, for liver diseases and diabetes, the stem cell therapy treatment approach is still in its infancy and further work is necessary. Many technical questions have yet to be answered before stem cell therapy can be applied to its fullest potential in the clinic. In order to achieve the goal of cell therapy, the source and types of cells, generation of cells in sufficient numbers, maintenance of the differentiated phenotype and cell engraftment and homing when transplanted in damaged tissue must be considered. Ideally, cells should expand extensively *in vitro*, have minimal immunogenicity and be able to reconstitute tissue when transplanted in damaged tissue. Defining which patient groups are suitable for this therapy and which stem cell types are the most effective given the underlying pathology is also important. The optimum timing and method of delivery need to be determined as they may have a significant influence on the outcome of cell transplantation. Long-term side-effects of treatment are unknown as most of the clinical studies are very recent. In addition, there is growing evidence that transplanted cells, being multipotent, do not simply replace missing tissue but also trigger local mechanisms to initiate a repair response. Paracrine effects and immune regulation of the transplanted cells may also play a role in functional restoration of the tissue (Pluchino et al. 2003).

As more scientific knowledge is gained in this field, hopefully some of the technical concerns will be answered and we will soon see stem cell therapy in more clinical applications.

References

- Alison MR, Poulson R, Jeffery R, Dhillon AP, Quaglia A, Jacob J, Novelli M, Prentice G, Williamson J, Wright NA (2000) Hepatocytes from non-hepatic adult stem cells. *Nature* 406:257
- am Esch JS 2nd, Knoefel WT, Klein M, Ghodsizad A, Fuerst G, Poll LW, Piechaczek C, Burchardt ER, Feifel N, Stoldt V, Stockschrader M, Stoecklein N, Tustas RY, Eisenberger CF, Peiper M, Haussinger D, Hosch SB (2005) Portal application of autologous CD133⁺ bone marrow cells to the liver: a novel concept to support hepatic regeneration. *Stem Cells* 23:463–470
- Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM (2002) Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 106:3009–3017
- Banerjee M, Kumar A, Bhonde RR (2005) Reversal of experimental diabetes by multiple bone marrow transplantation. *Biochem Biophys Res Commun* 328:318–325

- Bianco P, Riminucci M, Gronthos S, Robey PG (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells* 19:180–192
- Block GD, Locker J, Bowen WC, Petersen BE, Katyal S, Strom SC, Riley T, Howard TA, Michalopoulos GK (1996) Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SE, EGF and TGF alpha in a chemically defined (HGM) medium. *J Cell Biol* 132:1133–1149
- Bonner-Weir S, Weir GC (2005) New sources of pancreatic beta-cells. *Nat Biotechnol* 23:857–861
- Burns CJ, Persaud SJ, Jones PM (2004) Stem cell therapy for diabetes: do we need to make beta cells? *J Endocrinol* 183:437–443
- Choi JB, Uchino H, Azuma K, Iwashita N, Tanaka Y, Mochizuki H, Migita M, Shimada T, Kawamori R, Watada H (2003) Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. *Diabetologia* 46:1366–1374
- D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC (2004) Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. *J Cell Sci* 117:2971–2981
- Dahlke MH, Popp FC, Bahlmann FH, Aselmann H, Jager MD, Neipp M, Piso P, Klempnauer J, Schlitt HJ (2003) Liver regeneration in a retrorsine/CCl4-induced acute liver failure model: do bone marrow-derived cells contribute? *J Hepatol* 39:365–373
- Daneman D (2006) Type 1 diabetes. *Lancet* 367:847–858
- Devendra D, Liu E, Eisenbarth GS (2004) Type 1 diabetes: recent developments. *BMJ* 328:750–754
- Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429:41–46
- Ende N, Chen R, Reddi AS (2004) Effect of human umbilical cord blood cells on glycemia and insulinitis in type 1 diabetic mice. *Biochem Biophys Res Commun* 325:665–669
- Fallowfield JA, Iredale JP (2004) Targeted treatments for cirrhosis. *Expert Opin Ther Targets* 8:423–435
- Fang TC, Alison MR, Wright NA, Poulson R (2004) Adult stem cell plasticity: will engineered tissues be rejected? *Int J Exp Pathol* 85:115–124
- Fernandez Vina R, Andrin O, Saslavsky J, Ferreyra de Silva J, Vrsalovick F, Camozzi L, Ferreyra O, D Adamo C, Foressi F, Fernandez Vina R, Clasen A (2006a) Increase of 'c' peptide level in type 1 diabetics patients after direct pancreas implant by endovascular way of autologous adult mononuclear CD34+CD38(–) cells (Teceldiab 2 study). In: 4th International Society for Stem Cell Research Annual Meeting, Toronto
- Fernandez Vina R, Saslavsky J, Andrin O, Vrsalovick F, Ferreyra de Silva J, Ferreyra O, Camozzi L, Foressi F, D Adamo C, Fernandez Vina R (2006b) First word reported data from Argentina of implant and cellular therapy with autologous adult stem cells in type 2 diabetic patients (Teceldiar study 1). In: 4th International Society for Stem Cell Research Annual Meeting, Toronto
- Fiegel HC, Lioznov MV, Cortes-Dericks L, Lange C, Kluth D, Fehse B, Zander AR (2003) Liver-specific gene expression in cultured human hematopoietic stem cells. *Stem Cells* 21:98–104
- Fodor WL (2003) Tissue engineering and cell based therapies, from the bench to the clinic: the potential to replace, repair and regenerate. *Reprod Biol Endocrinol* 1:102
- Fogt F, Beyser KH, Poremba C, Zimmerman RL, Khettry U, Ruschoff J (2002) Recipient-derived hepatocytes in liver transplants: a rare event in sex-mismatched transplants. *Hepatology* 36:173–176
- Frankel MS (2000) In search of stem cell policy. *Science* 287:1397

- Gordon MY, Levičar N, Pai M, Bachellier P, Dimarakis I, Al-Allaf F, M'Hamdi H, Thalji T, Welsh JP, Marley SB, Davis J, Dazzi F, Marelli-Berg F, Tait P, Playford R, Jiao L, Jensen S, Nicholls JP, Ayav A, Nohandani M, Farzaneh F, Gaken J, Dodge R, Alison M, Apperley JF, Lechler R, Habib NA (2006) Characterisation and clinical application of human CD34⁺ stem/progenitor cell populations mobilised into the blood by G-CSF. *Stem Cells* 24:1822–1830
- Grompe M, Lindstedt S, al-Dhalimy M, Kennaway NG, Papaconstantinou J, Torres-Ramos CA, Ou CN, Finegold M (1995) Pharmacological correction of neonatal lethal hepatic dysfunction in a murine model of hereditary tyrosinaemia type I. *Nat Genet* 10:453–460
- Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* 401:390–394
- Halvorsen TL, Beattie GM, Lopez AD, Hayek A, Levine F (2000) Accelerated telomere shortening and senescence in human pancreatic islet cells stimulated to divide *in vitro*. *J Endocrinol* 166:103–109
- Heng BC, Yu H, Yin Y, Lim SG, Cao T (2005) Factors influencing stem cell differentiation into the hepatic lineage *in vitro*. *J Gastroenterol Hepatol* 20:975–987
- Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA, Bhatia M (2003) Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 21:763–770
- Ianus A, Holz GG, Theise ND, Hussain MA (2003) *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 111:843–850
- International Diabetes Federation (2006) Diabetes atlas. In: <http://www.eatlas.idf.org/>. Cited 23 Dec 2009
- Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ (2004) Hematopoietic stem cells convert into liver cells within days without fusion. *Nat Cell Biol* 6:532–539
- Kanazawa Y, Verma IM (2003) Little evidence of bone marrow-derived hepatocytes in the replacement of injured liver. *Proc Natl Acad Sci USA* 100 [Suppl 1]:11850–11853
- Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, Champlin RE, Estrov Z (2002) Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 346:738–746
- Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105:369–377
- Kucia M, Ratajczak J, Reza R, Janowska-Wieczorek A, Ratajczak MZ (2004) Tissue-specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury. *Blood Cells Mol Dis* 32:52–57
- Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat Med* 6:1229–1234
- Lammert E, Cleaver O, Melton D (2001) Induction of pancreatic differentiation by signals from blood vessels. *Science* 294:564–567
- Lechner A, Habener JF (2003) Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. *Am J Physiol Endocrinol Metab* 284:E259–E266
- Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL, Habener JF (2004) No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells *in vivo*. *Diabetes* 53:616–623

- Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, Chen JR, Chen YP, Lee OK (2004) *In vitro* hepatic differentiation of human mesenchymal stem cells. *Hepatology* 40:1275–1284
- Lee VM, Stoffel M (2003) Bone marrow: an extra-pancreatic hideout for the elusive pancreatic stem cell? *J Clin Invest* 111:799–801
- Li L, Xie T (2005) Stem cell niche: structure and function. *Annu Rev Cell Dev Biol* 21:605–631
- Mallet VO, Mitchell C, Mezey E, Fabre M, Guidotti JE, Renia L, Coulombel L, Kahn A, Gilgenkrantz H (2002) Bone marrow transplantation in mice leads to a minor population of hepatocytes that can be selectively amplified *in vivo*. *Hepatology* 35:799–804
- Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA (2004) Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. *Diabetes* 53:91–98
- Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR (2000) Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 290:1779–1782
- Michalopoulos GK, Bowen WC, Mule K, Luo J (2003) HGF-, EGF-, and dexamethasone-induced gene expression patterns during formation of tissue in hepatic organoid cultures. *Gene Expr* 11:55–75
- Ministry of Health (2001) Annual report of the chief medical officer. In: Ministry of Health Report, UK, London
- Misawa R, Ise H, Takahashi M, Morimoto H, Kobayashi E, Miyagawa S, Ikeda U (2006) Development of liver regenerative therapy using glycoside-modified bone marrow cells. *Biochem Biophys Res Commun* 342:434–440
- Miyazaki M, Akiyama I, Sakaguchi M, Nakashima E, Okada M, Kataoka K, Huh NH (2002) Improved conditions to induce hepatocytes from rat bone marrow cells in culture. *Biochem Biophys Res Commun* 298:24–30
- Moriscot C, de Fraipont F, Richard MJ, Marchand M, Savatier P, Bosco D, Favrot M, Benhamou PY (2005) Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation *in vitro*. *Stem Cells* 23:594–603
- Najimi M, Sokal E (2005) Liver cell transplantation. *Minerva Pediatr* 57:243–257
- Ng IO, Chan KL, Shek WH, Lee JM, Fong DY, Lo CM, Fan ST (2003) High frequency of chimerism in transplanted livers. *Hepatology* 38:989–998
- Oh SH, Miyazaki M, Kouchi H, Inoue Y, Sakaguchi M, Tsuji T, Shima N, Higashio K, Namba M (2000) Hepatocyte growth factor induces differentiation of adult rat bone marrow cells into a hepatocyte lineage *in vitro*. *Biochem Biophys Res Commun* 279:500–504
- Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM, Petersen BE (2004) Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. *Lab Invest* 84:607–617
- Ohashi K, Park F, Kay MA (2001) Hepatocyte transplantation: clinical and experimental application. *J Mol Med* 79:617–630
- Okumoto K, Saito T, Hattori E, Ito JI, Adachi T, Takeda T, Sugahara K, Watanabe H, Saito K, Togashi H, Kawata S (2003) Differentiation of bone marrow cells into cells that express liver-specific genes *in vitro*: implication of the Notch signals in differentiation. *Biochem Biophys Res Commun* 304:691–695
- Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001) Bone marrow cells regenerate infarcted myocardium. *Nature* 410:701–705

- Pessina A, Eletti B, Croera C, Savalli N, Diodovich C, Gribaldo L (2004) Pancreas developing markers expressed on human mononucleated umbilical cord blood cells. *Biochem Biophys Res Commun* 323:315–322
- Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP (1999) Bone marrow as a potential source of hepatic oval cells. *Science* 284:1168–1170
- Petersen KF, Shulman GI (2006) Etiology of insulin resistance. *Am J Med* 119:S10–S16
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147
- Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U, Amadio S, Bergami A, Furlan R, Comi G, Vescovi AL, Martino G (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* 422:688–694
- Preston SL, Alison MR, Forbes SJ, Direkze NC, Poulosom R, Wright NA (2003) The new stem cell biology: something for everyone. *Mol Pathol* 56:86–96
- Ratajczak MZ, Kucia M, Reza R, Majka M, Janowska-Wieczorek A, Ratajczak J (2004) Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells ‘hide out’ in the bone marrow. *Leukemia* 18:29–40
- Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A (2000) Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat Biotechnol* 18:399–404
- Ruhnke M, Ungefroren H, Nussler A, Martin F, Brulport M, Schormann W, Hengstler JG, Klapper W, Ulrichs K, Hutchinson JA, Soria B, Parwaresch RM, Heeckt P, Kremer B, Fandrich F (2005) Differentiation of *in vitro*-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. *Gastroenterology* 128:1774–1786
- Ryan EA, Lakey JR, Paty BW, Imes S, Korbitt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AM (2002) Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157
- Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H, Okita K (2004) Transplantation of bone marrow cells reduces CCl₄-induced liver fibrosis in mice. *Hepatology* 40:1304–1311
- Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM (2002) Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 109:1291–1302
- Sell S (2001) Heterogeneity and plasticity of hepatocyte lineage cells. *Hepatology* 33:738–750
- Sipione S, Eshpeter A, Lyon JG, Korbitt GS, Bleackley RC (2004) Insulin expressing cells from differentiated embryonic stem cells are not beta cells. *Diabetologia* 47:499–508
- Taneera J, Rosengren A, Renstrom E, Nygren JM, Serup P, Rorsman P, Jacobsen SE (2006) Failure of transplanted bone marrow cells to adopt a pancreatic beta-cell fate. *Diabetes* 55:290–296
- Tang DQ, Cao LZ, Burkhardt BR, Xia CQ, Litherland SA, Atkinson MA, Yang LJ (2004) *In vivo* and *in vitro* characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes* 53:1721–1732
- Terai S, Sakaida I, Yamamoto N, Omori K, Watanabe T, Ohata S, Katada T, Miyamoto K, Shinoda K, Nishina H, Okita K (2003) An *in vivo* model for monitoring trans-differentiation of bone marrow cells into functional hepatocytes. *J Biochem (Tokyo)* 134:551–558
- Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, Yokoyama Y, Uchida K, Yamasaki T, Fujii Y, Okita K, Sakaida I (2006) Improved liver function in liver cirrhosis patients after autologous bone marrow cell infusion therapy. *Stem Cells* 24:2292–2298

- Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS (2000a) Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* 31:235–240
- Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS (2000b) Liver from bone marrow in humans. *Hepatology* 32:11–16
- Till JE, McCulloch E (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14:213–222
- Vig P, Russo FP, Edwards RJ, Tadrous PJ, Wright NA, Thomas HC, Alison MR, Forbes SJ (2006) The sources of parenchymal regeneration after chronic hepatocellular liver injury in mice. *Hepatology* 43:316–324
- Wagers AJ, Sherwood RI, Christensen JL, Weissman IL (2002) Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297:2256–2259
- Wang X, Ge S, McNamara G, Hao QL, Crooks GM, Nolte JA (2003) Albumin-expressing hepatocyte-like cells develop in the livers of immune-deficient mice that received transplants of highly purified human hematopoietic stem cells. *Blood* 101:4201–4208
- Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H (2004) Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 364:141–148
- Wu T, Ciepły K, Nalesnik MA, Randhawa PS, Sonzogni A, Bellamy C, Abu-Elmagd K, Michalopoulos GK, Jaffe R, Kormos RL, Gridelli B, Fung JJ, Demetris AJ (2003) Minimal evidence of transdifferentiation from recipient bone marrow to parenchymal cells in regenerating and long-surviving human allografts. *Am J Transplant* 3:1173–1181