CHAPTER 34

HSCT for tissue regeneration in adults

F. Prosper

1. Introduction

The development of pluripotent human embryonic stem cells (1) and the isolation of different types of stem cells derived from adult tissues (somatic and germinal) have raised the possibility of utilising these cells for non-haematological diseases. Among the different tissue-specific stem cells, some adult-derived stem cells, such as haematopoietic cells, have been endowed with a remarkable plasticity, suggesting that they may be able to contribute to regeneration of multiple tissues. An intense controversy has emerged as to whether observations supporting the plasticity of adult stem cells represent true pluripotentiality or may be explained by other mechanisms such as cell fusion, reprogramming or just a misinterpretation of certain experiments or even an excess of enthusiasm. Although from a biological perspective these are legitimate issues, the most topical question currently is whether these stem cells can be exploited clinically for non-haematological diseases in a similar way to the haematological disorders. The importance of understanding the mechanism underlying the potential benefit of stem cells for tissue regeneration remains critical.

2. Bone marrow derived stem cells

The bone marrow is a remarkable reservoir of adult stem cells. Beside HSC and the side population (SP) cells, other non-haematopoietic populations of stem cells have been isolated from the bone marrow including mesenchymal stem cells (MSC) (also known as marrow stromal cells), multipotent progenitor cells (MAPC), multipotent stem cells and pre-MSC, MIAMI cells (Marrow-Isolated Adult Multilineage Inducible cells), VSEL cells (Very Small Embryonic Like stem cells) or endothelial progenitor cells (EPCs). The main characteristics of these different populations of BM-derived cells are shown in Table 1. Whether all these populations truly represent completely different types of cells or whether their differences are due to the techniques of isolation and characterisation is unclear. The potential of these cells is also under intense debate. Only HSC and MSC have been exploited in clinical trials of regenerative therapy for non-haematological diseases, while other cell populations have been studied in different experimental settings.

3. Cell therapy with stem cells from the bone marrow

Multipotent adult stem cells obtained from human BM are just starting to be explored in numerous diseases and for different tissues. This Chapter will discuss those areas in which stem cell therapy has advanced the furthest. Overall, evidence for efficacy is limited to a few applications including epidermal layers, transplantation of limbo-corneal stem cells or chondrocytes for cartilage lesions. In most diseases only preclinical studies and initial phase I/II studies have been completed so that a large effort is still needed before cell therapy becomes a reality.

Table 1: **Bone marrow derived stem cells**

Name	Species	Tissue	Phenotype	ESC genes	Meso	Endo	Ecto
MAPC	Human Mouse, rat, swine	BM, Brain Muscle	CD44 low, MHC-I low, CD45 -, Thy1-, cKit+	Oct4+ Nanog-	+	+	+
MIAMI	Human	BM	CD44 Pos; MHC-I Neg, CD45 Neg	Oct4+ Nanog?	+	+	+
VSEL	Mouse Human	BM UCB	CD45-, Lin-, Sca1 +, CXCR4 +, SSEA1+	Oct4+ Nanog+	+	+	+
Pre-MSC	Mouse	BM	SSEA1+, CD45-, Ter119- , Sca1 Dim	Oct4+ Nanog+	+	+	+
MASC	Human	BM, Liver, Heart	CD45-	Oct4+ Nanog+	+	+	+
MSC	Human Mouse, rat, swine	BM, Fat UCB	CD44+, MHC-I+, CD45-, Thy1-, cKit+	Oct4- Nanog-	+	+?	+?

3.1. Stem cells for cardiovascular repair

The idea of repairing the diseased heart using different types of stem cells has gained widespread attention recently, leading not only to a number of preclinical studies but also to early phase I/II and even randomised clinical trials (2). In contrast to ESC, there is no convincing evidence that cells from postnatal tissues other than the heart can generate functional cardiomyocytes *in vivo*. Nevertheless, a number of cell populations, including total BMMNC, enriched haematopoietic stem cells, EPCs, BM, MSC and adipose MSC and skeletal myoblasts have been grafted in models of chronic as well as acute myocardial infarction (MI) (3). Improved function has been seen in a number of studies without evidence that the cells contributed to cardiac muscle itself. As a consequence of these preclinical studies, clinical trials in patients with acute and chronic myocardial ischaemia using mostly BM cells (4) (freshly isolated total BM, mononuclear cells, cultured cells to increase putative endothelial progenitor cells, CD34 or AC133 selected cells) have been developed. The largest experience has been gained in patients with acute MI using BM-MNC and while some randomised trials suggest a benefit others find no effect (5). The differences have been attributed to the heterogeneity of the types of cells used in each trial, the type of patients and/or the endpoints.

BM-MNC include an heterogeneous pool of cells such as stem cells (at very low frequency), progenitor cells and mature cells. Transplantation of either pure selected populations or unselected populations each have a rationale and with the current level of knowledge both approaches are justified. Although there is much to learn in this field, some lessons are emerging. The classic idea that delivery of the appropriate cells would repair a damaged heart via active myocardial regeneration resulting from trans-differentiation of the administered cells has been superseded by the recognition of alternative mechanisms of action:

- exogenous cells may stimulate proliferation of endogenous cardiac precursors or stem cells through neovascularisation or paracrine signalling actions facilitating the ability of the heart to heal itself;
- exogenous cells may lead to cardiac repair via fusion of donor cells with host cardiomyocytes as has been demonstrated in animal models;
- the effect of cells could also be mediated by altering the mechanical properties of the scar thereby preventing deterioration in cardiac function (5).

Peripheral vascular disease (PVD) is another potential target for regenerative medicine. Approximately 15% of adults over the age of 55 have detectable haemodynamic impairments with intermittent claudication and critical limb ischaemia being the two major clinical presentations. Experimental studies indicate that infusion of progenitor cells from different sources such as peripheral blood or BM may improve recovery after limb ischaemia. Based on these experimental findings, clinical phase I trials were initiated in 2001 (6). These initial pilot trials indicated that infusion bone marrow-derived or circulating blood-derived progenitor cells improves the blood supply to the legs in patients with ischaemia resulting in longer pain-free walking distances (7). These initial studies need to be confirmed on a larger scale and with clear endpoints.

3.2. Orthopaedic applications of stem cells

On the basis of the *in vitro* observation that MSCs can differentiate into osteocytes and chondrocytes, BM-derived MSCs were seeded onto extracellular matrices such as hydroxyapatite and then implanted *in vivo* into NOD/SCID mice or in animals with segmental bone defects and new bone formation was observed (8). BM-derived MSC have been transplanted into mice with *osteogenesis imperfecta*, a genetic disorder of mesenchymal tissues. In addition BMT had been used in children with this disease. The cells engrafted with an increase in the number of osteoblasts, formation of new lamellar bone and an increase in the total body mineral content were observed. Clinically there was a reduction in the frequency of fractures and enhancement of the body growth rate (9).

Particularly promising for orthopaedic applications, especially for bone formation, is the use of natural or synthetic biomaterials as carriers for MSC delivery. Newer materials such as biodegradable polymers poly-L-lactide (PLA) and poly-L-lactideco-glycolide (PLGA) can facilitate adhesion, proliferation and differentiation of cells. Clinical studies have been designed in which scaffolds were loaded with *in vitro* expanded autologous BM-derived MSCs and successfully implanted in patients with large bone defects. More recently, an extended mandible discontinuity was successfully repaired through heterotopic bone induction with biomaterials, autologous BM cells and growth factors (10).

3.3. Stem cells for diabetes

Replacement of insulin-producing cells in patients with diabetes has become a major goal in regenerative therapy. Promising strategies have included either the use of cells with the potential to generate insulin-producing β-cells by expansion of existing, β-cells, differentiation of embryonic stem (ES) cells to β-cells, conversion of either pancreatic or non-pancreatic adult stem/progenitor cells into β-cells or the use pharmacological agents that seeks to regenerate β-cells in the pancreas, either by replication of existing β-cells (regeneration) or by the generation of new β-cells from other cell types (neogenesis). Numerous studies have been undertaken to establish whether insulin-producing β-cells can be developed from stem-progenitor cells from different origins. The initial claims that bone marrow stem cells could differentiate into other lineages, including insulin-producing cells are at best questionable at this time (11). It is possible however that BM cells may contribute to the endothelium of damaged pancreas, leading to the production of cytokines and growth factors that stimulate β-cell neogenesis, β-cell proliferation, or increased survival of the residual β-cells (12).

3.4. CNS disorders

Diseases of the CNS, although a very attractive target for cell therapy, represent a significant challenge for cell-based strategies of repair, due to the complexity of the interactions between different types of cells and the required need for integration of the different structures. During prenatal development of the mammalian CNS, the neural stem cells (NSCs) and their progenitors expand and give rise to the functional neurons and glial cells that constitute the growing brain. A number of studies during the last 15 years have clearly demonstrated that in the adult mammalian CNS, a small number of NSCs are able to self-renew and generate different neural cell lineages including neurons, astrocytes and oligodendrocytes under specific microenvironmental stimuli (13).

As we have described in other diseases, recent studies have suggested that terminal neural differentiation can also be seen with non-CNS-derived multipotent somatic stem cells, such as BM-derived stem cells. Most of these studies are based on the potential of non-neural stem cells to acquire certain “neural” markers *in vitro* after treatment with certain cytokines or factors that induced neural differentiation. However, studies have only recently started to address whether putative neural progeny from MSCs or other stem cells have functional characteristics consistent with neurons. There is no substantial evidence for *in vivo* differentiation and functional integration of any of these BM-derived stem cells. Regardless of the lack of true differentiation capacity, a number of studies in experimental models suggest that BM-derived stem cells like MSC or EPC can contribute to improve the functional capacity in patients with multiple sclerosis, stroke or spinal cord injuries (14).

Acknowledgments

Supported in part by grants from the Government of Navarra, Ministerio de Sanidad, European Framework Project 6 and FEDER funds and the University of Navarra.

References

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998; 282: 1145-1147.
2. Laflamme MA, Murry CE. Regenerating the heart. Nat Biotechnol 2005; 23: 845-856.
3. Murry CE, Field LJ, Menasche P. Cell-based cardiac repair: Reflections at the 10-year point. Circulation 2005; 112: 3174-3183.
4. Wollert KC, Drexler H. Clinical applications of stem cells for the heart. Circ Res 2005; 96: 151-163.
5. Dimmeler S, Burchfield J, Zeiher AM. Cell-Based Therapy of Myocardial Infarction. Arterioscler Thromb Vasc Biol 2007.
6. Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: A pilot study and a randomised controlled trial. Lancet 2002; 360: 427-435.
7. Lenk K, Adams V, Lurz P, et al. Therapeutical potential of blood-derived progenitor cells in patients with peripheral arterial occlusive disease and critical limb ischaemia. Eur Heart J 2005; 26: 1903-1909.
8. Petite H, Viateau V, Bensaid W, et al. Tissue-engineered bone regeneration. Nat Biotechnol 2000; 18: 959-963.
9. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999; 5: 309-313.
10. Warnke PH, Springer IN, Wiltfang J, et al. Growth and transplantation of a custom vascularised bone graft in a man. Lancet 2004; 364: 766-770.
11. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 2003; 111: 843-850.
12. Bonner-Weir S, Weir GC. New sources of pancreatic beta-cells. Nat Biotechnol 2005; 23:

857-861.
13. Quinones-Hinojosa A, Sanai N, Soriano-Navarro M, et al. Cellular composition and cytoarchitecture of the adult human subventricular zone: A niche of neural stem cells. J Comp Neurol 2006; 494: 415-434.
14. Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat Med 2004; 10 Suppl: S42-50.

Multiple Choice Questionnaire

To find the correct answer, go to http://www.esh.org/ebmt-handbook2008answers.htm

1. BM contains different populations of stem cells. One of the following is not a BM derived population of stem cells:

a) Mesenchymal stem cells ☐
b) Haematopoietic stem cells ☐
c) Endothelial progenitor cells ☐
d) Myoblasts ☐

2. Transplantation of BM derived stem cells in patients with acute MI:

a) Is a well established and effective therapy ☐
b) Has demonstrated that HSC differentiate into functional cardiomyocytes *in vivo* ☐
c) Phase III clinical trials suggest that it may be associated with a functional benefit ☐
d) All of the above ☐

3. Mesenchymal stem cells have been used in patients with one of the following diseases:

a) Parkinson's Disease ☐
b) Peripheral vascular disease ☐
c) Stroke ☐
d) Osteogenesis imperfecta ☐

4. Pluripotentiality is a characteristic of:

a) Haematopoietic stem cells ☐
b) Side population ☐

c) Endothelial progenitor cells ☐
d) Embryonic stem cells ☐

---

5. Which of the following markers are expressed by pluripotent stem cells:

a) Oct4 ☐
b) CD34 ☐
c) Sox2 ☐
d) Both a and c ☐