Bone marrow stromal cell : mediated neuroprotection for spinal cord repair

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Chapter 1

Introduction

Introduction Part A

Stem Cells for Central Nervous System Repair and Rehabilitation

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ABSTRACT

The central nervous system (CNS) has limited capacity for self-repair. Current treatments are often incapable of reversing the debilitating effects of CNS diseases that result in permanent and/or progressive physical and cognitive impairments. One promising repair strategy is transplantation of stem cells, which can potentially replace lost neurons and/or glia or promote repair through secretion of trophic factors. Various types of stem cells exist, each with their own advantages and disadvantages. Although no consensus exists regarding the optimal cell type to use, moderate functional improvements have been shown in animal models of CNS diseases using different types of stem cells. However, the precise mechanism of action behind their beneficial effects remains unknown. In addition, many barriers to clinical use still need to be resolved before transplantation of stem cells can be used as effective biologics. These barriers include—depending on the stem cell type—possible tumor formation, difficulty with harvest, limited in vivo differentiation and integration, and ethical issues regarding use.

INTRODUCTION

Central nervous system (CNS) diseases are often characterized by complex immune-mediated cytotoxic and apoptotic processes that result in the loss of function and permanent loss of neural cells[1]. Although many CNS diseases result from a loss of viable cells, a therapeutic approach must consider the type of cell lost to have a beneficial outcome. For example, Parkinson disease requires replacement of lost dopaminergic substantia nigra neurons, whereas multiple sclerosis requires reconstitution of functional oligodendrocytes. Stem cells have the potential to address this demand for specific cells for specific diseases because of their multipotency, and thus stem cell therapy is a promising biologic therapy to consider for persons with CNS diseases.

In the past decade an explosive amount of stem cell research has been conducted, resulting in an insightful scope of knowledge on stem cell biology. Continuing research will be essential before effective bedside treatments for CNS diseases may be developed. This review aims to provide a critical overview of stem cell use for repair of CNS diseases relevant for rehabilitation medicine.

DEFINITION, ORIGIN, AND VARIOUS TYPES OF STEM CELLS

By definition, a stem cell is capable of self-renewal and of differentiating into at least one other cell type. The zygote is referred to as a totipotent stem cell. The blastocyst contains an inner cell mass consisting of self-replicating cells that can become all but trophoblast cells (the outer layer blastocyst cells that later become the placenta); these cells are known as pluripotent stem cells (Figure 1). When these cells enter into 1 of the 3 primary germ layers—ectoderm, mesoderm, or endoderm—they are referred to as multipotent stem cells. These cells then can become precursor cells, which are unipotent cells that differentiate into the final cell types within differentiated tissues (Figure 1).

When stem cells are harvested from embryonic tissue, they are considered embryonic stem cells (ESC). When stem cells are taken from tissues from the adult body, they are

referred to as adult stem cells (ASC [note: adipose tissue-derived stem cells are sometimes also referred to as ASC]) or somatic stem cells. The existence of ASC was first demonstrated within the adult hematopoietic system, which throughout natural life gives rise to new blood cells[2]. After this discovery, ASC were demonstrated within numerous other adult tissues such as neural stem cells (NSC) in the brain, epidermal neural crest stem cells (EPI-NCSC) in hair follicles, muscle-derived (mesenchymal) stem cells in muscles, and bone marrow stromal cells (BMSC) in bone marrow. The functions of ASC are poorly understood, but one rational possibility would be that ASC support repair of the tissues in which they reside. At present, however, this theory has not been confirmed unequivocally, and it certainly does not appear to be the case in the CNS, where endogenous restoration is poor and disease or trauma typically elicits permanent damage.

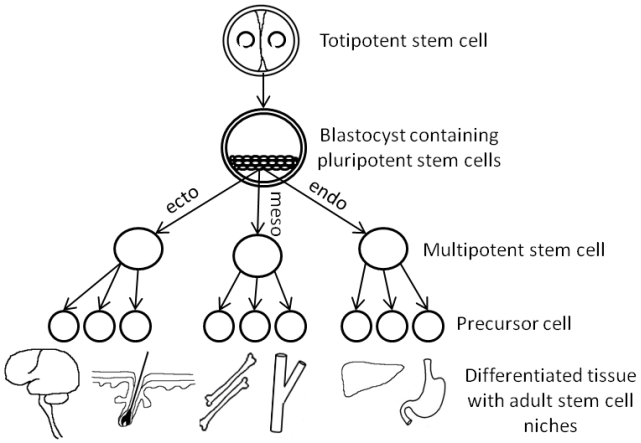

Fig. 1. Hierarchy of stem cells. Totipotent cells can develop into all cell types of the body, pluripotent cells can become all but trophoblast cells, multipotent cells can give rise to all cells within 1 of the 3 germ layers, and precursor cells are unipotent cells that will become terminally differentiated cells of specialized tissue. Ecto = ectoderm germ layer; meso = mesoderm germ layer; endo = endoderm germ layer.

Recently, a third type of stem cell has emerged—the induced pluripotent stem (iPS) cell, discovered by Takahashi and Yamanaka in 2006[3]. The iPS cell is generated from an adult somatic cell by introducing transcriptional factors whose ectopic expression reprograms the cell into a pluripotent cell. The groundbreaking discovery 'that mature cells can be reprogrammed to become pluripotent' has earned Yamanaka the Nobel Prize in

Physiology or Medicine 2012. The prize was shared with John B. Gurdon who in 1962 used an enucleated oocyte into which the nucleus of an adult cell was transferred to create a stem cell capable of forming a blastula and eventually a tadpole[4]. In June 2013 the somatic cell nuclear transfer method was for the first time successfully used for human embryonic stem cell generation[5]. The discovery of generated stem cells is opening exciting new avenues in the field of regenerative medicine (for review, see Bellin et al.[6]).

UTILITY OF NEURAL AND NON-NEURAL STEM CELLS

NSC can contribute in different ways to repair of the brain and spinal cord. They can potentially differentiate into neurons and/or glial cells and replace those that were lost as a result of the disease or trauma. Alternatively, NSC can serve as vectors for growth factors that could support cell survival, cell proliferation, axon regeneration, and blood vessel formation, which can all positively influence CNS repair. It is also possible that stem cells serve as a substrate for regenerating axons and thus contribute to repair. Thus far, numerous studies have demonstrated the potential of stem cells for CNS repair. Interestingly, the mechanisms underlying their benefits remain elusive.

Embryonic NSC have a robust capacity to differentiate into neural cells and are therefore suitable for repair strategies based on cell replacement. However, their impressive differentiation capacity comes with uninhibited proliferation, which could result in tumor formation after transplantation. This factor, together with ethical concerns surrounding their harvest, has limited the application of ESC for CNS repair. Adult NSC also are capable, albeit less so than ESC, of differentiating into neural cells, and in contrast to ESC, they are not known for causing tumors after transplantation. Thus adult NSC are good candidate cells for neural replacement approaches. A disadvantage of adult NSC is that they are difficult to obtain because they need to be harvested from the adult brain or spinal cord.

Compared with embryonic and adult NSC, non-neural stem cells are more readily obtainable. For instance, BMSC reside in bone marrow, EPI-NCSC in hair follicles, and muscle-derived (mesenchymal) stem cells in muscles, and all these tissues are relatively

easy to harvest from adults. Some of these non-neural stem cells offer additional advantages such as the low expression of major histocompatibility complex I molecules by BMSC that would help evade immunologic rejection. Importantly, it was reported that several types of non-neural stem cells could (trans)differentiate into neural cells[7], which has opened new avenues for CNS repair. However, at present, this potential to become a neuron, astrocyte, or oligodendroglial cell has not been unambiguously proven and is in fact a subject of controversy. If this ability to transdifferentiate into neural cells is low or absent, their benefits in replacement strategies would be poor. On the other hand, non-neural stem cells may offer effective means to repair the CNS through their ability to secrete repair-supporting molecules such as growth factors. Moreover, in accordance with their decreased differentiation capacity, these cells are less inclined to unrestrained proliferation and are therefore less tumorigenic. Table 1 provides an overview of the relative advantages and disadvantages of different types of stem cells. The current controversies and challenges within the field of regenerative medicine are best illustrated with the following example. For a mesodermally derived BMSC to be suitable for CNS repair based on cell replacement, it will need to transdifferentiate into a neuron or neural glial cell. For this transdifferentiation to occur, the BMSC will first need to revert into a pluripotent cell, subsequently differentiate into an ectodermal precursor cell, and

Table 1. Relative advantages and disadvantages of embryonic and adult stem cells for human use in CNS disease

	ESC	ASC		iPS
		NSC	MSC	
Differentiation/cell replacement potential	Very good	Good	Poor	Good
Harvest	Controversial	Difficult	Easy	Easy
Tumorigenicity	High	Low	Low	High

Abbreviations: ESC, embryonic stem cell; ASC, adult stem cell; NSC, neural stem cell; MSC, mesenchymal stem cell; iPS, induced pluripotent stem cell

then differentiate into a neuron, astrocyte, or oligodendrocyte. Several studies have shown that BMSC can be induced in vitro to express neuronal markers and even to have some electrical neuronal properties, but true transdifferentiation into a fully functioning neuron is strongly debated[8]. Similarly, a few in vivo studies[7,9] have shown expression of neuronal markers and/or anatomic integration after transplantation of BMSC, but neuronal functionality (ie, synapse formation, firing of action potentials, and release of neurotransmitters) or glial functionality has not been shown unequivocally. For example, Kopen and colleagues (1999)[7] reported the expression of glial fibrillary acidic protein, a marker for astrocytes, in BMSC after transplantation into mice brain ventricles and concluded that they had transdifferentiated into mature astrocytes. The expression of specific neural markers is an important first step toward applying BMSC for CNS cell replacement, but it appears to be a rare event, and it is not a demonstration that the cell has become a functional component of the nervous system.

ANATOMIC AND FUNCTIONAL REPAIR AFTER STEM CELL TRANSPLANTATION IN ANIMAL MODELS OF CNS DISEASE

In the past decade a number of studies showed that transplantation of NSC can result in histologic and/or functional improvements in rodent models of various CNS diseases. Cummings and colleagues[10] demonstrated remyelination of axons and functional improvements after transplantation of NSC into a mouse spinal cord injury model. Improved motor function was observed in hemiplegic mice after implantation of monkey ESC[11]. Table 2 provides a selected overview of studies that have transplanted neural and non-neural stem cells in different CNS disease models and reported repair. Typically, in ESC transplantation paradigms, some degree of differentiation into neurons and glia is shown. From the results it appears that NSC preferentially differentiate into astrocytes. Some may also differentiate into oligodendrocytes, but very few differentiate into neurons. Whether these newly generated neural cells then integrate within the host CNS tissue is not always clearly demonstrated. Despite the alleged in vitro ability, it is not often reported that non-neural stem cells become neural cells after transplantation into the CNS. Nevertheless, anatomic and/or functional repair has been demonstrated. Sieber-

Blum[12] showed improvements in sensory connectivity and in touch perception after transplantation of EPI-NCSC in a mouse spinal cord injury model. In this study it was proposed that the neural crest cell–derived EPI-NCSC have the advantages of ESC and ASC because they are able to differentiate into oligodendrocytes and neuroblasts without being tumorigenic and are easily obtained from the bulge of hair follicles.

Table 2. Selected overview of studies that have implanted stem cells in rodent models of spinal cord injury, stroke or Parkinson's disease

Type of stem cell	Disease Model	Differentiation	Functional Outcome	Reference
ESC	SCI, rat	Astrocytes, oligo's, neurons	↑BBB	McDonald et al.[36]
Fetal NPC	SCI, rat	Neurons	↑Pellet retrieval	Ogawa et al.[37]
BMSC	SCI, rat	-	↑BBB	Hofstetter et al. 2002[21]
NSC	SCI, mouse	Neurons, oligo's	↑BBB	Cummings et al.[10]
EPI-NCSC	SCI, mouse	Neurons, oligo's	↑Touch perception	Sieber-Blum[12]
iPS	SCI, mouse	Neurons, oligo's, astrocytes	↑BMS	Tsuji et al.[13]
ESC	Stroke, mouse	Neurons	↑Beam walking, ↑rotarod	Ikeda et al.[11]
BMSC	Stroke, rat	Oligo's, astrocytes	↑body swing test, ↑footprint analysis	Chen et al.[9]
NSC	Stroke, rat	Neurons, astrocytes	-	Kelly et al.[38]
BMSC	Parkinson, rat	-	↑Rotational behavior	Wu et al.[15]

Abbreviations: ESC, embryonic stem cell; SCI, spinal cord injury; oligo's, oligodendrocytes; BBB, Basso, Beattie, and Brasnahan-scale; NPC, neural progenitor cell; BMSC, bone marrow stromal cell; NSC, neural stem cell; EPI-NCSC, epidermal neural crest stem cells; iPS, induced pluripotent stem cell.

Another cell type that has putative neural differentiation capacity without being tumorigenic is the iPS cell. The therapeutic potential of iPS cells was nicely demonstrated in a mouse spinal cord injury model, which revealed that transplanted iPS cell–derived neurospheres differentiated into all 3 neural cell types, participated in remyelination, promoted axonal outgrowth, and improved locomotor function[13]. Additionally, this study circumvented tumor formation by pre-evaluation and selection of the neurospheres for tumorigenicity[13]. This study was partially confirmed by Nutt and colleagues, who showed successful transplantation and integration of iPS cell-derive NPCs into an early chronic spinal cord injury model, however, without evidence of functional improvement.[14]

IMPROVING THE OUTCOME AFTER STEM CELL TRANSPLANTATION INTO THE DAMAGED CNS

Notwithstanding recent reports that transplanted stem cells can become neural cells, the key mechanism for functional improvements observed after ASC transplantation in the CNS is thought to be neuroprotection, that is, limiting the loss of tissue. Neuroprotection can be accomplished through the secretion of growth factors, such as brain-derived neurotrophic factor, glial cell line–derived neurotrophic factor, and nerve growth factor. A cell transplant provides long-term delivery of growth factors, which is an important advantage over direct injection, because growth factors dilute rapidly and typically have short half-lives. For this reason, recent studies have genetically engineered stem cells to overexpress such growth factors, with the aim of enhancing their neuroprotective capacity and, as a result, their repair-supporting potential. Wu and colleagues[15] showed a neuroprotective effect of glial cell line–derived neurotrophic factor-overexpressing BMSC in a Parkinson model. Axonal regeneration and enhanced functional recovery was found after transplantation of brain-derived neurotrophic factor-overexpressing BMSC in a spinal cord injury model[16].

The beneficial effects of stem cells can also be increased by pre-differentiating the cells in vitro prior to transplantation. This pre-differentiation can be achieved by growing the cells according to a particular induction protocol that pushes the cells into a desired lineage.

Davies and colleagues[17] showed improved axon regeneration and locomotor function in rats with spinal cord injuries after transplantation of astrocytes differentiated from embryonic glial-restricted precursors, but not undifferentiated glial-restricted precursors. Hofstetter and co-workers[18] transduced NSC with neurogenin-2 to suppress astrocytic differentiation prior to transplantation into rats with spinal cord injuries and demonstrated prevention of graft-induced sprouting, decreased allodynia, and improved functional recovery. Although these 2 studies achieved some degree of restoration with use of opposite differentiation protocols, it is clear that both demonstrated that the repair-supporting abilities of stem cells can be positively influenced prior to transplantation.

Another way to improve the outcome is by combining stem cell transplantation with putative additive or synergistic treatments. Even though combinatorial strategies are thought to be essential to achieve biologically significant repair, exploration of these strategies has been sparse. Combining BMSC transplantation with an inhibitor of Rho-kinase[19], a molecule known to prevent neurite outgrowth, or with olfactory ensheathing cells[20], another adult cell type that has been shown to benefit anatomic and functional CNS repair, did not improve functional outcomes in rats with spinal cord injuries more than BMSC transplantation alone. In a similar model, the combination of BMSC with physical exercise did not improve function compared with control subjects[21]. On the other hand, in both a spinal cord injury[22] and a stroke[23] model, the outcome after transplantation of brain-derived adult NSC with olfactory ensheathing cells was improved compared with transplantation of each of the cell types alone. In a rat model of cerebral ischemia, the combination of BMSC with erythropoietin showed a synergistic effect on neurogenesis and memory performance but not on locomotor function[24]. Because of our limited current knowledge about mechanisms underlying single treatments, it is difficult to select the appropriate combinations in which the single interventions would exert additive or synergistic effects. Future elucidation of mechanisms will allow more rationally targeted combinatorial repair strategies.

It is clear that, despite some promising results, stem cell–based repair of the damaged CNS still has major challenges to overcome before it can be successfully applied in a clinical setting. These challenges vary between tumorigenic and ethical concerns with ESC[25], differentiation issues with ASC[26,27], and survival of transplanted stem cells in general[28]. Poor survival of stem cells after injection into damaged CNS tissue can be due to poor vascularization of the transplantation site or a result of inflammation with accompanying secretion of cytotoxic molecules and rejection by activated immune cells. To optimally benefit from transplanted stem cells, it will be necessary to develop strategies to improve survival of the cells after transplantation. Although concomitant treatment with immunosuppressive drugs[27], improving the timing of cell transplantation[28], and transplantation of cells within a scaffold all have been shown to benefit cell survival, the majority of cells still die within weeks of transplantation. Clearly, further studies that focus on optimizing stem cell survival after transplantation into the damaged CNS are imperative.

CLINICAL TRIALS

Despite our incomplete knowledge, several clinical trials are currently being conducted in which stem cells are being transplanted in patients with Parkinson disease, stroke and other neurological disorders, with variable results thus far. After transplantation of human fetal mesencephalic tissue in patients with Parkinson disease, grafted neurons have been reported to survive and integrate, with improvements in several outcome parameters. These improved parameters included a 37% reduction in Levodopa dose, a 40% improvement in 18F-Flurodopa uptake (a measurement of dopaminergic activity in the putamen), a 44% improvement in the Unified Parkinson Disease Rating Scale Motor Score (while being off medication), a 39% decrease in “off medication” time, and a 49% decrease in “on medication” time with dyskinesia, according to a meta-analysis[29].

Impressively, up to sixteen years after transplantation, dopaminergic innervations in basal ganglia could be restored to normal levels and was associated with relief of motor symptoms.[30] Variability across studies, however, is high. The differences between the observations in different clinical trials are likely due to variances in recipient

characteristics (eg, younger patients seem to show better recovery after neural grafting), the use of different surgical techniques, and/or (lack of) immunosuppressive drug administration, resulting in decreased graft survival.

The first clinical trial using human ESC for spinal cord injury was approved in 2009 by the United States Food and Drug Administration (FDA). In this Phase I trial, oligodendrocyte progenitor cells derived from human ESCs were safely transplanted into five severe spinal cord injury patients. In November 2011 however, the trial was discontinued for financial reasons.[31] Another Phase I/II trial by StemCells Inc using human CNS stem cells for spinal cord injury is currently underway in Switzerland and Canada and has recently (October 2013) been approved by the FDA[32]. Small clinical trials in other neurologic diseases, including stroke[33] and Huntington disease[34], seem to support the potential use of stem cells, because moderate functional improvements are being achieved in some patients. For example, after transplantation of neuronal cells in 12 patients who have sustained a basal ganglia stroke, 6 patients showed improvements on the European stroke scale (a gain of 3 to 10 points) 12 to 18 months after transplantation[33]. Transplantation of fetal neural tissue in 5 patients with Huntington disease resulted in cognitive improvements in 3 patients the first 2 years after surgery, which then faded after 4-6 years, as measured by the Unified Huntington Disease Rating Scale and neuropsychological tests. Safety was shown up to ten years postoperatively[35]. Several other clinical trials are in or have completed phase I/II of safety, but large trials of efficacy of stem cells for neurological disorders are still lacking.

CONCLUSION

Transplantation of stem cells can potentially be used for treatment of various CNS diseases. Progress is being made in the laboratory, and in various animal models of CNS disease/disorders, moderate functional improvements are being reported. The underlying mechanisms are still mostly unclear. In addition, determination of which stem cell type would be best for a particular CNS disease/disorder is still largely unresolved. Clearly many issues need to be elucidated before safe and effective stem cell-based therapies can be

designed for bedside treatments of neurologic disorders. All these issues warrant further investigations before stem cells can live up to their potential as effective biologic treatments for CNS disease.

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