Chapter 5
Stem Cell-Based Therapies for Parkinson's Disease

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5.1 Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder that is especially common in the elderly. It results from the progressive death of pigmented dopaminergic neurons (DAn) in the *substantia nigra pars compacta* (SNpc) that project axons to the striatum where dopamine is released (Buttery and Barker 2014; Martínez-Morales and Liste 2012; Lindvall and Kokaia 2009). This provokes a reduction in striatal dopamine levels, causing most of the clinical motor symptoms, which include tremors, rigidity, bradykinesia and other debilitating symptoms (Savitt et al. 2006).

It is now known that the pathology extend far beyond the nigrostriatal dopaminergic pathway itself, as other dopaminergic and non-dopaminergic systems are also affected in PD. Accordingly, in addition to the better-known motor symptoms, PD patients suffer several nonmotor symptoms. These symptoms can include sleep disturbances, dementia and mood disturbances which can have a significant impact on the quality of life of patients (Lindvall 2016).

The etiology of PD is still not fully understood. However, some possible pathogenic mechanisms have been proposed such as impairment of mitochondrial function and trophic support, abnormal action of kinases, excessive release of oxygen free radicals and dysfunction of protein degradation (Wang et al. 2015).

Besides death of DAn, other hallmarks of PD include the presence of protein aggregates made up of α-synuclein-positive Lewy bodies in several brain regions, as well as neuro-inflammation causing the disease progression (Wang et al. 2015; More et al. 2013; McGeer and McGeer 2008). Indeed, activation of microglial and glial cells and inflammatory responses are common features of both animal models

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of PD and PD patients, playing a significant role in the neurodegenerative progression of DAn (Wang et al. 2015; Hirsch et al. 2012).

Today, there is still no cure for PD. However, a variety of treatment options are available to help relieve motor symptoms, which can greatly improve the quality of life of the patients.

Current treatments include: deep brain stimulation (DBS) in the subthalamic nucleus or globus pallidus. This treatment option **co**nsists of placing a stereotactically guided microelectrode into a specific brain structure. A stimulator is also implanted into the chest of the patient, adjusting the stimulation parameters by telemetry. DBS is a safe and reversible procedure that modulates specific targets in the brain resulting in motor symptom improvement. DBS is especially effective managing long-term motor complications resulting from L-dopa treatment, such as dyskinesias and wearing-off phenomena. However, the technique still needs to be optimized in order to increase efficiency, as the procedure is expensive and not all patients are equally likely to improve (Larson 2014; Heumann et al. 2014)

Available pharmacological therapies aim to increase dopamine levels in the brain by providing dopaminergic agonists, or inhiting dopamine breakdown (catechol-O-methyl transferase and monoamine oxidase inhibitors) (Lindvall 2016; Prashanth et al. 2011). The most common treatment for PD is levodopa (L-dopa). L-dopa is able to cross the blood brain barrier and once in the brain is transformed into dopamine by dopaminergic neurons.

Previously described treatments are effective in alleviating some symptoms during early phases of the disease, but long-term efficacy is unknown. Furthermore, these treatments are associated with certain side effects, including motor fluctuations, on-off phenomena and involuntary movements as dyskinesias. Ultimately, none of these treatments are reparative, and do not stop the disease from progressing (Lindvall 2016; Poewe 2009; Politis et al. 2014).

Cell-replacement clinical trials based on transplantation of human fetal mesencephalic tissue, a tissue rich in dopaminergic neuroblasts, have provided *proof of principle* that cell replacement therapy can work in the human PD brain (Barker et al. 2013; Björklund and Dunnett 2007). In the most successful trials, DAn generated from the transplanted tissue was able to re-innervate the denervated striatum and become functionally integrated, restoring the striatal DA release and giving rise to clear symptomatic relief in some patients (Kefalopoulou et al. 2014; Petit et al. 2014; Piccini et al. 1999).

However, ethical and practical aspects related to tissue availability limit their widespread clinical use. It is therefore necessary to seek alternative cell sources, mainly based on the use of stem cells. Due to their properties, stem cells are now considered best candidates as an alternative to DAn, different from fetal mesencephalic tissue.

Human DA precursors have been efficiently derived from different sources of stem cells including: human Embryonic Stem Cells (hESCs) (Kriks et al. 2011; Cho et al. 2008; Chambers et al. 2009; Malmersjö et al. 2010), human induced Pluripotent Stem Cells (hiPSCs) isolated from control, or from PD patients (Soldner et al. 2009; Hargus et al. 2010; Nguyen et al. 2011; Sánchez-Danés et al. 2012), human Neural Stem Cells (hNSCs) both from fetal (Courtois et al. 2010; Villa et al. 2009) or adult

brains (Lévesque et al. 2009) human Mesenchymal Stem Cells (hMSCs) by the induction with different cytokines and neurotrophic factors like GDNF (Kitada and Dezawa 2012; Trzaska and Rameshwar 2011; Dezawa et al. 2004). More recently, it has been shown the direct conversion of fibroblasts into functional "induced" DAn (iDA). Still has to be explored to what extent these cells can contribute to functional recovery in models of PD (Caiazzo et al. 2011; Pfisterer et al. 2011).

In this chapter, we discuss some general issues related to the clinical use of stem cells to treat Parkinson's disease. We describe the different types of stem cells available nowadays, their properties and how they are being developed and applied in PD patients.

5.2 Grafts of Human Fetal Ventral Mesencephalic Tissue

The initial idea for cell transplantation in PD was simple: in theory, adult DAn lost by neurodegenerative processes could be replaced by immature human DAn (Brundin et al. 1988).

It all started between the 1970s-1980s, several different groups demonstrated that DAn obtained from the fetal ventral mesencephalon (VM) were able to survive and integrate into the host tissue, release dopamine and enhance motor function in animal models of PD.

Similar results were obtained with mesencephalic xenografts transplanted in the striatum of rats under immunosuppression (Brundin et al. 1988). The promising results from these experimental trials then allowed several groups to perform open label clinical trials in PD patients.

Cell replacement therapies (CRT) for PD have been conducted during the last 30 years using different source of cells. The most effective cells have so far been allogenic fetal ventral mesencephalic tissue grafts, which contains developing midbrain DAn and their precursors.

In general transplants are done in the striatum, the region where project their axons dopaminergic neurons of SNpc. Successful open-label trials reported improved motor symptoms in a number of patients (Barker et al. 2013; Björklund and Dunnett 2007; Freed et al. 1992; Spencer et al. 1992; Widner et al. 1992; Lindvall et al. 1990), improved ^{18}F –DOPA uptake (Piccini et al. 1999; Lindvall et al. 1994; Peschanski et al. 1994) and robust long-term graft survival lasting over a decade as shown by postmortem analysis, even though some grafted cells showed Lewy body formation (Hallett et al. 2014; Kordower et al. 2008; Li et al. 2010).

Additionally, the grafted tissue re-innervated the host striatum and became functionally integrated into the recipient circuitry (Kefalopoulou et al. 2014; Petit et al. 2014; Piccini et al. 1999). However, two placebo-controlled trials showed modest benefit in the first end point analyzed (Freed et al. 2001; Olanow et al. 2003). Furthermore a group of patients developed graft-induced dyskinesia that persisted even in the absence of L-dopa treatment (Hagell et al. 2002). Fortunately, a later evaluation of these grafts by F-DOPA uptake and UPDRS scores, showed statistically significant improvement of fetal tissue transplantation for certain patients, in line with reports from open-label trials (Barker et al. 2013; Freed et al. 2011).

The reasons behind the inconsistency of these results may be related to poor standardization procedures, including patient selection, tissue preparation, immunosuppression procedures, primary endpoints, and general trial design. In any case, all these trials have revealed several limitations of the procedure for routine clinical practice that should be improved in order to develop a viable cell replacement therapy (CRT) for PD (Olanow et al. 2003; Isacson et al. 2003; Ganz et al. 2011). The main challenges are related to ethical issues related to the use of fetal tissue, poor standardization of the tissue dissection and cell material processing. This last limitation can contribute to the appearance of dyskinesia (related in part, to the serotonergic component of the graft), be related to the high variability in graft survival and be associated with inconsistent clinical benefit (Carta et al. 2008; Politis et al. 2010). Another important limitation is the host immunological and inflammatory response, since autologous tissue cannot be used (Rath et al. 2013; Piquet et al. 2012; Arenas 2010; Piccini et al. 2005).

There are several problems associated with performing such transplants. First of all it is extremely difficult to obtain sufficient amounts of fetal tissue, as each patient requires tissue obtained from between 4 and 10 aborted fetuses that must also have the adequate embryonic age. Currently there is no good method of cryopreservation; the mesencephalic tissue can be maintained at 4 °C for 1 week, but the quality of the tissue decreases with longer storage times. Furthermore, due to the need to mix cell suspensions from different donors, it is complicated to control HLA system; therefore it is difficult to standardize the quality of the donor cells.

Currently, the European consortium TRANSEURO (www.transeuro.org.uk) (see Table 5.1) is making significant efforts to optimize the design of clinical trials for CRT in PD. The main objective of this project is to develop a safe and effective method for treating PD patients using fetal VM cells that can serve as a model for future clinical trials.

This trial is designed to minimize technical variables such as: patient selection (age, type of PD), tissue preparation and collection, graft placement and support, immunosuppressive treatment, follow up time and quantifiable endpoints. Therefore, this study can serve as a reference to develop future stem-cell transplantation assays and will provide a more coherent view of the current therapeutic improvement (Abbott 2014; Moore et al. 2014; Gonzalez et al. 2015a).

5.3 Stem Cell Properties and Requirements for Their Application in CRT for PD

As mentioned above, a major challenge in developing cell transplantation into a routine clinical practice for PD is the deficiency of fetal tissue supply, which implies the use of several fetal tissue to treat a single patient. For this reason, important research efforts have been carried out to find alternative sources of cells for

Table 5.1 Human stem cells used in clinical trials for treatment of Parkinson's disease

Stem cell type	Transplant type	Delivery administration	Status	Sponsor
hMSCs from bone marrow	Allogenic	Intravenous administration	Phase 1 NCT02611167	The University of Texas Health Science Center
	Autologous	Intravenous administration	Phase 1/2 NCT01446614	Guangzhou General Hospital of Guangzhou Military Command
Adipose-Derived hMSCs	Autologous	into the Vertebral Artery and Intravenously	Phase 1/2 NCT01453803	Ageless Regenerative Institute
	Autologous	Not provided	Recruiting NCT02184546	StemGenex
hNSCs from fetal ventral mesencephalic tissue	Allogenic	Intracerebral implantation	Phase 1 (TransEuro Project) NCT01898390	University of Cambridge
	Allogenic	Not provided	Phase 1/2 NCT01860794	Bundang CHA Hospital
	Allogenic	Intracerebral implantation	NCT02538315	University of Saskatchewan
hNSCs from adult cerebral cortex	Autologous	Intracerebral implantation to the left putamen	Phase 0 NCT01329926	NeuroGeneration (Lévesque et al. 2009)
Human parthenogenetic-derived NSCs	Allogenic	Intracerebral implantation to the striatum and Substantia Nigra	Phase 1 NCT02452723	Cyto Therapeutics Pty Limited

Abbreviations: *hMSCs* human mesenchymal stem cells, *hNSCs* human neural stem cells

transplantation in PD. Several cell sources have been explored in order to generate DAn. The most promising cells found so far are stem cells.

Stem cells are undifferentiated cells characterized by their ability to proliferate and differentiate into more specialized types of cells. Stem cells can be classified according to how they were obtained or by their differentiation potential. Based on their ability to differentiate, stem cells are divided basically into two major categories: pluripotent stem cells (which can give rise to specialized cells of the three germ layers, i.e. endoderm, mesoderm and ectoderm) and multipotent stem cells (more specialized cells, that can generate specific cell lineages of a particular germ layer, although recently it has been shown that some multipotent cells possess the capacity to transdifferentiate into cells of more than one germ layer, such as MSCs) (Bongso et al. 2008; Zhan and Kilian 2013; Macias et al. 2010).

Overall it is assumed that in order to make the differentiation of DAn from stem cells a clinically competitive treatment option for PD, these cells need to be equivalent to those of human VM tissue in terms of their phenotype, as well as neurochemical and electrophysiological properties both *in vitro* and *in vivo* after grafting.

This means that transplanted cells must induce a substantial improvement of motor symptoms, without causing side effects (Lindvall et al. 2012; Martínez-Serrano and Liste 2010). To achieve this, grafted cells must survive, re-innervate the striatum, integrate into the neural circuitry of the host and exhibit the same characteristics of authentic nigral A9 DAn.

Also they have to satisfy a number of safety requirements such as not forming tumors, avoiding the development of dyskinesia, either by the presence of serotonergic neurons or inappropriate distribution of implants, and they should not induce immune rejection in the host. Furthermore, it must be possible to grow sufficient numbers of these cells in order to reach clinical relevance. As a result, only a reduced number of clinical trials are being conducted in which stem cells are applied.

In addition to CRT itself, stem cells can also be beneficial by providing a trophic support, by improving the survival of affected neurons (Lindvall and Kokaia 2009; Lunn et al. 2011) or acting as inflammation modulators. Not surprisingly, both epidemiological and genetic studies support an important role of neuro-inflammation in the pathophysiology of PD (Hirsch et al. 2012; More et al. 2013).

5.4 Multipotent Stem Cells

5.4.1 Human Neural Stem Cells

Human Neural Stem Cells (hNSCs) are uncommitted and multipotent cells with the ability for self-renewal that can differentiate into all the neural cells of CNS (i.e. neurons, astrocytes and oligodendrocytes). These cells can be obtained from differentiation of pluripotent stem cells and from fetal, neonatal, and adult brain.

In the human brain, NSCs are found in the developing nervous system and in two neurogenic niches of the adult brain, the subventricular zone of the lateral ventricles and the dentate gyrus in the hippocampal formation (Kempermann et al. 1997). However their regeneration potential is very limited and there is no evidence of endogenous neurogenesis in the SN in PD.

It has been shown that these cells are also present in the subventricular zone of PD patients and they are able to proliferate, which could provide a future possibility to develop novel therapeutic approaches to stimulate these cells to migrate to the striatum and differentiate into dopamine neurons (Van den Berge et al. 2011, 2013). However the number of proliferating cells is small and highly variable between

individuals and methods to expand this population of cells for clinical use remain to be established.

It is also worth noting that adult neurogenesis may be affected during the progression of PD. In fact, it has been observed that the proliferation of cells appears to be decreased in the brains of PD patients. Therefore, an alternative option may involve infusing exogenous hNSCs to replacing lost neurons (Höglinger et al. 2004).

hNSCs can be propagated *in vitro* as free-floating aggregates, called neurospheres. Neurospheres are a mixture of NSCs and progenitor cells grown in the presence of growth factors such as basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF) (Bonnamain et al. 2012; Kallur et al. 2006). An alternative approach for the expansion of hNSCs is the genetic immortalization where cells are transduced with immortalizing genes (e.g., TERT, v-Myc or c-Myc) and supporting the proliferation by adding growth factors (Villa et al. 2009; Cacci et al. 2007).

Several types of hNSCs have been explored in order to generate midbrain DAn, including human VM neural precursors. These, in theory, should be the ideal candidates for cell therapies in PD.

Unfortunately, VM neural precursors present poor growth potential and unstable phenotypes losing their initial properties with repeated passages in culture (Villa et al. 2009; Ramos-Moreno et al. 2012). Furthermore, they survive poorly into the brain when grafted (Kim et al. 2009a), limiting them from being a stable source of human DAn.

Recently an efficient method to generate large numbers of midbrain DAn has been described. This method is based in the expansion and differentiation of neural precursor cells present in the human VM tissue by adding Wnt5a. Using this method, a sixfold increase of the number of midbrain DAn was obtained as compared to the starting VM preparation (Ribeiro et al. 2013), and could solve in part, the lack of VM tissue in future transplantation studies.

A different tactic consists of immortalizing hNSCs from the VM, although it can not be considered for clinical use. If these methodologies are successful, they could end some of the limitations described previously (Villa et al. 2009; Liste et al. 2004; Lotharius et al. 2002).

Despite all of this, an efficient method to induce midbrain dopamine neurons from NSCs in large numbers for clinical treatment is still lacking.

In a different approach, hNSCs isolated from brain biopsies have been used to treat PD patients by the company Neuro Generation Inc. In this trial, biopsied cortical and subcortical tissue samples during craniotomy were proliferated for several months and then differentiated into neurons (60 % GABAergic, 15 % DAn) and glial cells. These cells were implanted in multiple sites in the putamen. Patients showed some motor improvement, increased dopamine uptake and other clinical benefits (Lévesque et al. 2009). Further trials are being developed to determine the feasibility and efficiency of this method (see Table 5.1 and Fig. 5.1).

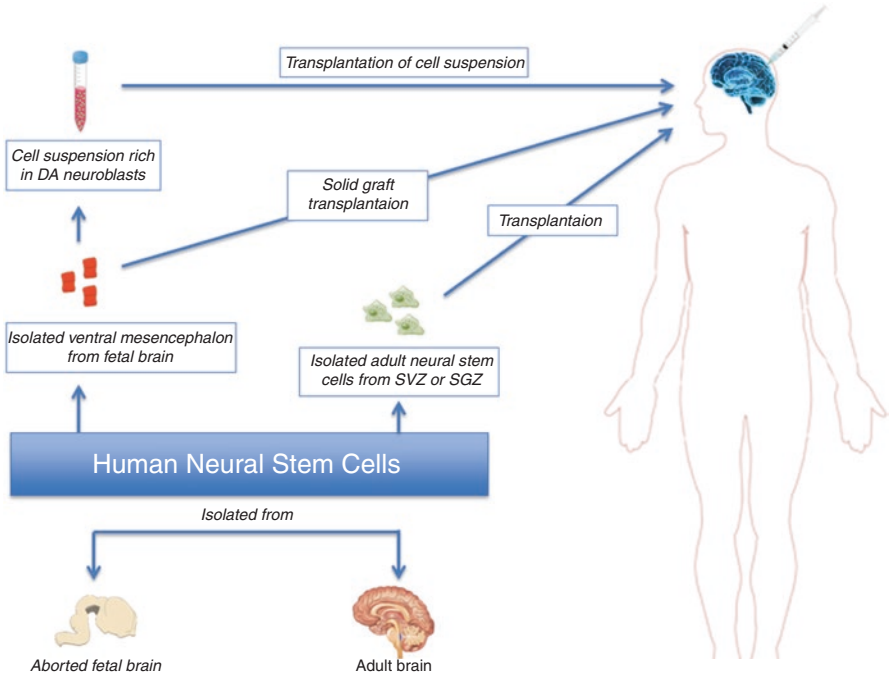

Fig. 5.1 Schematic representation of the possible use of human Neural Stem Cells for Cell Replacement Therapy (CRT) for treatment of PD

5.4.2 Human Mesenchymal Stem Cells

Human Mesenchymal Stem Cells (hMSCs), also named marrow stromal cells, may be an alternative source of multipotent stem cells that, until now, have primarily been isolated from adult bone marrow (Collins et al. 2014). However, these cells can also be found elsewhere in the body, including adipose tissue (Zuk et al. 2002), umbilical cord blood (Erices et al. 2000), dental pulp (Gronthos et al. 2000), placenta (Abumaree et al. 2013) and brain (Paul et al. 2012).

Their widespread availability throughout the body, in addition to their great proliferative potential once isolated, has made MSCs emerge in the last years as a promising approach in regenerative medicine (Teixeira and Carvalho 2013; Ryan et al. 2005).

hMSCs are stromal cells characterized by the adherence to plastic in cell culture. They exhibit positive expression of specific markers such as CD105, CD73 and CD90, and they do not express hematopoietic markers like CD34, CD45, HLA-DR, CD14, CD11B or CD19. They also show multi-lineage differentiation potential to cells of mesodermal origin (osteocytes, chondrocytes, adipocytes) (Lunn et al. 2011; Trounson and Pera 2001; Joyce et al. 2010). Recently, some evidence has shown that they can transdifferentiate towards a neural lineage

(Macias et al. 2010; Lunn et al. 2011; Satija et al. 2009; Paul et al. 2012) and even obtain a DAn phenotype (Trzaska and Rameshwar 2011; Dezawa et al. 2004; Paldino et al. 2014). Therefore, it has become plausible to use these stem cells for the generation of DA-like neurons.

In vivo studies in macaque models of PD after transplantation of dopamine producing cells induced from autologous bone-marrow derived MSCs, have proved very promising.

However, beneficial effects were not conclusively shown to be caused by dopaminergic neuron integration, but rather could be caused by neurotropic effects of the MSCs (Hayashi et al. 2013).

Another recent study using primed-fetal liver MSCs showed functional and neurochemical recovery of dopaminergic neuron activity in a 6-OHDA mouse model when transplanted directly into the striatum. However, it was also shown that not all primed MSCs differentiated into a neuron-like cell. Therefore, further studies are needed for long-term efficacy and safety before considering this cell source for CRT in humans (Kumar et al. 2016).

Furthermore, Chun et al., showed a potentially viable source of MSC-derived dopaminergic neurons differentiated from dental pulp *in vitro*, confirming morphological identity and dopamine release. The results from this study need to be taken further to verify if the cells possess the electrophysiological characteristics necessary for proper integration *in vivo* (Chun et al. 2016).

Still, the differentiation potential of hMSCs is much more limited than that of pluripotent stem cells. The trans-differentiation of MSCs from a mesodermal to a neural cell lineage is still relatively new in the field, and for this reason their use as a source for CRT in neurodegenerative disorders remains somewhat controversial. The studies that have been done show inconsistent and inconclusive results in animal models and, it is still unsure if these neuron-like cells derived from MSC can be correctly integrated into the host-neural circuitry to form synaptic connections (Joyce et al. 2010; Hardy et al. 2008; Glavaski-Joksimovic and Bohn 2013). For these reasons, more research needs to be done and better standardization procedures of MSC sources, along with improved differentiation protocols are needed to produce viable and consistent dopaminergic-like neurons before being used in clinical trials.

Currently, the discovery of the beneficial effects mediated by the hMSCs secretome (a concept defined as the proteins secreted by cells or tissues that become crucial for the regulation of subsequent cellular processes) may be a more promising approach to their use as a treatment option for PD (Teixeira and Carvalho 2013). MSCs secrete protective neurotrophic factors, growth factors and cytokines that promote protection, repair and show immunomodulatory effects. Additionally, other advantage of the use of hMSCs is that they could, in theory, circumvent the need of immunosupression in cell therapies, as they can be derived from patient own tissue. For this same reason, they are not ethically controversial when compared to hESCs, adding another reason to their growing popularity as a cell source for treatment options in several different diseases, including PD.

Paracrine factors excreted by MSCs have been shown to modulate the plasticity of host tissue, by secreting neurotrophic and growth factors such as vascular endo-

thelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF-1) (Hoch et al. 2012), brain derived neurotrophic factor (BDNF), β-nerve growth factor (β –NGF), transforming growth factor β (TGF- β), fibroblast growth factor 2 (FGF2), and glial cell derived neurotrophic factor (GDNF) which are involved in increasing neurogenesis, promoting neuronal and glial cell survival, angiogenesis, inhibition of apoptosis, immunomodulation and showing neuroprotective actions in brain (Siniscalco et al. 2010; Chamberlain et al. 2007; Teixeira and Carvalho 2013). In addition, MSCs produce extracellular matrix proteins (ECM) that could favor neural cell attachment, growth and neuritogenesis that could lead to functional neural restoration (Tate et al. 2010; Li et al. 2010).

Several studies have shown that both naïve bone marrow-derived MSCs (BM-MSCs) and neurally differentiated BM-MSCs had therapeutic effects in PD animal models. This was verified because of their capacity to regenerate and protect damaged DAn. MSCs isolated from the umbilical cord, adipose tissue and placenta have shown neuroprotective and neuro-regenerative effects in PD animal models too (McCoy et al. 2008; Mathieu et al. 2012; Park et al. 2012). Currently, MSCs isolated from adipose tissue are receiving more attention because of easier access and differentiation potential as compared to other sources of MSCs (Chang et al. 2014). In addition to the studies showing neuroprotective effects of grafted MSCs, a recent study also showed that MSCs derived specifically from adipose tissue showed a modulatory role after transplantation into the medial forebrain bundle, supporting their ability to enhance endogenous neurogenesis and adapt to a noxious microenvironment that could making them a potentially safe treatment option for PD (Schwerk et al. 2015).

Although the exact pathogenesis of PD is unknown, mounting evidence is suggesting that chronic neuronflammation is one of the causes of pathophysiology of PD. The presence of activated astrocytes and microglia leads to neurodegeneration and loss of DA neurons, making it a good treatment target by increasing cytokines such as TNFB, IL-4 and IL 10 (Wang et al. 2015). Following these findings, studies have shown that MSCs not only act through paracrine effects relying on close cell-contact to the injured area, but release soluble factors that can be involved in immunosuppression in the brain (Kim et al. 2009b) and inhibit the release of pro-inflammatory cytokines (Ng et al. 2014).

Another property of hMSCs is they are able to migrate to places of injury in animals when they are infused systemically, suggesting their migratory potential and promoting the repair process through the secretion of growth factors, cytokines, and antioxidants (Teixeira and Carvalho 2013; Vegh et al. 2013). Migration can also be induced by growth factors and chemokines released after damage that could provide migratory signals that induce activation of integrins from MSCs and up-regulation of selectins, promoting cells to interact with the endothelium, similar to leukocytes of the immune system (Martínez-Morales et al. 2013; Teo et al. 2012)

Recently, another study also confirmed the beneficial effects of combined treatment of MSC conditioned medium and neural stem cell grafting, showing behavioral and functional improvement in a PD animal model (Yao et al. 2015).

Although it is believed that all MSCs generally possess the same regenerative properties, populations isolated from different tissues are biologically heterogeneous

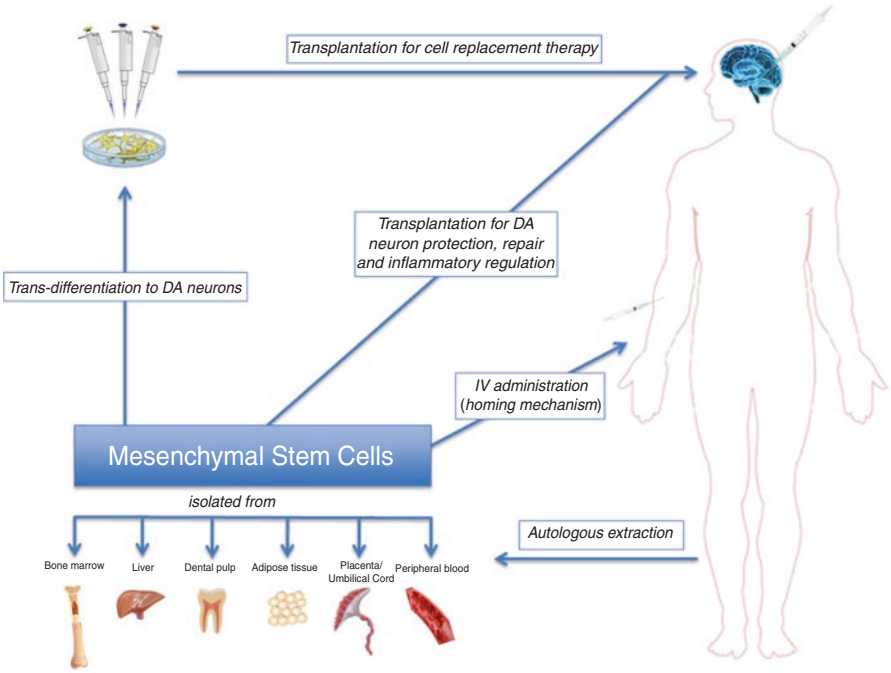

Fig. 5.2 Mesenchymal stem cells can be isolated from different tissues and infused intracerebrally or systemically for treating Parkinson´s Disease (PD) patients

and may vary in their immune-phenotype, proliferation rate and commitment to different cell lines (Paul and Anisimov 2013). Therefore, their use in clinical trials has been limited. However, an open label study from 2009 using bone marrow derived MSCs in seven patients showed immediate and short term safety to use these cells for treatment of PD, but the clinical improvement they displayed were only marginal (Venkataramana et al. 2009).

Furthermore, hMSCs from adipose tissue and bone marrow are currently being used to investigate the efficacy of autologous and allogenic treatments in PD patients with the idea of taking advantage of their immune-modulatory and trophic properties (Kitada and Dezawa 2012; Schwarz and Storch 2010]) (Table 5.1 and Fig. 5.2).

5.4.3 Human Pluripotent Stem Cells

In theory, pluripotent stem cells are the ideal material for the treatment of several diseases using cell therapy, mainly due to their ability to self-renewal and to differentiate into any cell type of the body.

However there are still several risks related to their use: the possibility of tumor formation, host immune reactions, technical questions related to correct

differentiation with the desired phenotype and ethical issues (in the case of human embryonic stem cells). Currently, the use of hPSCs is extremely regulated in most countries, leading to a reduced number of approved clinical trials involving their use.

5.4.4 Human Embryonic Stem Cells

The first human Embryonic Stem Cells (hESCs) were isolated from the inner cell mass of the blastocyst, in 1998 (Thomson et al. 1998). These cells are characterized by their self-renewal capacity and the potential to differentiate towards specialized cells of all germ layers (endoderm, ectoderm and mesoderm). Due to these properties, hESCs can be a suitable cell source for cell-based therapies.

The clinical application of hESCs in neurodegenerative diseases such as PD depends on their efficient differentiation into the DAn phenotype. In this regard, functional VM DAn have been obtained using different protocols. Currently, the most effective ones are those based in dual SMAD inhibition (Grealish et al. 2014; Kirkeby et al. 2012, 2013) and conversion of hESCs into precursors of floor plate that, after exposure to agonists of Shh and Wnt signaling pathways, are efficiently converted to DAn (Kriks et al. 2011; Chambers et al. 2009). In both cases, neurons generated can survive and integrate into the lesioned brain with long-term functional benefits, encouraging the research aimed at using hESCs for treating PD.

The recent work of Malin Parmar's group has demonstrated the DAn derived from hESCs can survive and innervate the brain regions of interest after grafting into the brain of parkinsonian rats (Grealish et al. 2014). Demonstrating that cell transplantation of hESC-derived DAn are functionally comparable to that of neurons derived from fetal tissue.

However, the use of hESCs is still associated with several problems including ethical issues, phenotype instability, controlling cell proliferation and correct differentiation and maturation into the desired phenotype (Petit et al. 2014; Cho et al. 2008; Martínez-Morales et al. 2013).

Furthermore to optimize the use of hESCs in clinical application, it is essential to avoid any risk of contamination, including xenogenic contamination (from cell culture reagents or feeder cells). This implies that neuronal precursors and neurons differentiated from hESCs must be obtained in GLP/GMP (Good Laboratory and Manufacture Procedures) conditions from the blastocyst isolation.

Besides, the work with hESCs is being extensively regulated in most countries. The guidelines ranging from controlled permissiveness to absolute prohibition (Condic and Rao 2010).

Finally, another challenge is the possibility of graft rejection. However, this problem could be avoided by the creation of banks of cells from immunologically diverse donor cells to include a high diversity of HLA types. Due to these limitations no clinical trial has been conducted with these cells in PD yet. But maybe not for long, as there are already planned at least two clinical trials with hESCs, one in Europe and another in the USA, to begin in the next few years (Abbott 2014).

5.4.5 Human Parthenogenetic Embryonic Stem Cells

The parthenogenetic cells are pluripotent stem cells obtained from unfertilized oocytes by the suppression of the second meiotic division, generating diploid cells carrying only the maternal chromosomes (Barker et al. 2016; Revazova et al. 2007). These cells are named parthenogenetic human embryonic stem cells (phESCs), show similar morphology to hESCs, express the typical pluripotency markers and show high levels of activity for telomerase and alkaline phosphatase (Revazova et al. 2007). They also generate embryoid bodies *in vitro* and teratoma formation after infusion in immunodeficient mice (Revazova et al. 2007).

Parthenogenetic human embryonic stem cells could be a good alternative to hESCs derived by somatic nuclear transfer (SCNT) since the process of parthenogenesis is relatively simple as compared to SCNT and does not require complex equipment (Gonzalez et al. 2015b; Revazova et al. 2007). These cells are not fertilized or activated via sperm entry and they can circumvent the ethical issues related to the use of hESCs.

However the lack of one parental contribution makes them different from hESC or hiPSCs. For this reason, their clinical use could be problematic, as it could affect the cell cycle and their ability to differentiate properly.

The first clinical trial using human pluripotent stem cells to treat PD was approved at the end of 2015 by the Australian government. The study will be carried out at the Royal Melbourne Hospital in Melbourne, Australia by the company International Stem Cell Corporation (ISCO). This is a Phase 1/2 trial in twelve patients with PD. For transplantation, the company is planning to use a population of NSCs previously generated from the parthenogenetic pluripotent stem cells (Barker et al. 2016).

5.4.6 Human Induced Pluripotent Stem Cells

The first ES-like cells from adult somatic cells were generated by Takahashi and Yamanaka through the overexpression of a few transcription factors (Takahashi and Yamanaka 2006). These ES-like cells were generated by transducing mouse embryonic fibroblasts (MEFs) with retroviruses that expressed Oct3/4, Sox2, Klf4, and c-Myc (abbreviated as OSKM). The combination of the four transcription factors gave rise to the known "induced pluripotent stem cells (iPSCs)" (Takahashi and Yamanaka 2006).

A year later it was reported the generation of human iPSCs from fibroblasts by two different laboratories, Yamanaka's team (Takahashi et al. 2007) and Thomson' s group (Yu et al. 2007). The first one used OSKM factors, while the second included NANOG and LIN28.

Human iPSCs are similar to hESCs in many aspects like morphology, expression of pluripotency factors, epigenetic marks, differentiation potential *in vivo* and *in*

vitro and the ability to generate viable chimeras (Martínez-Morales and Liste 2012; Phanstiel et al. 2011).

Certainly the cell reprogramming technology and the ability to generate iPS cells has been a breakthrough in the field of biomedical and clinical research. Since these cells can be used as *in vitro* models of diseases and for autologous grafting (no immunosuppression should be necessary). Further, they do not generate ethical controversies as can be derived from adult tissues.

Focusing in PD, human DA precursors have been efficiently derived from hiPSCs isolated from control, or from PD patients by using similar protocols to those used for differentiation of hESCs (Kriks et al. 2011; Soldner et al. 2009; Hargus et al. 2010; Nguyen et al. 2011; Sánchez-Danés et al. 2012; Kirkeby et al. 2012). When transplanted into the brain of lesioned rats, hiPSCs were able to survive and differentiate into DAn, showing a significant improvement in the motor tests, without generating tumors (Doi et al., 2014).

However we must be careful, since iPS cells have, at least, the same challenges and risks as hES cells; in addition to the problems associated with the reprogramming process itself and that they can be derived from the tissue of patients (they will carry the same genetic defects as the patient cells).

In fact, most efficient strategies for hiPSCs generation are based in the use of retroviral and lentiviral vectors that can integrate into the somatic cell genome, increasing the risk of oncogenic transformation and/or insertional mutagenesis. Currently, to avoid these risks, important advances have been made in the field by using excisable vectors (Soldner et al. 2009), non-integrative vectors (Stadtfeld et al. 2008), the use of direct protein or mRNA delivery (Bernal 2013; Zhou et al. 2009) and the addition of different chemical compounds (Masuda et al. 2013).

Some studies have identified genomic instability, as well as epigenetic and genetic abnormalities associated with the reprogramming process itself (which could be expected because most of the reprogramming factors used possess oncogenic potential (Revilla et al. 2015; Pasi et al. 2011; Lister et al. 2011). Another obstacle to overcome in hiPS-based cell technology before its therapeutic application is the risk of tumor formation. This risk is associated in part with the existence of proliferating cells after implantation but also with the phenotype heterogeneity of the differentiated cells as it happens in hESCs (Fu and Xu 2012; Thomson et al. 1998; Ben-David and Benvenisty 2011).

In any case, the expectations placed on hiPSCs are huge and several clinical trials are already planned with these cells for PD waiting government approval. One is expected to start soon in Japan led by the group of Jun Takahashi (Morizone and Takahashi 2016; Drouin-Ouellet and Barker 2014; Garber 2013) (Fig. 5.3).

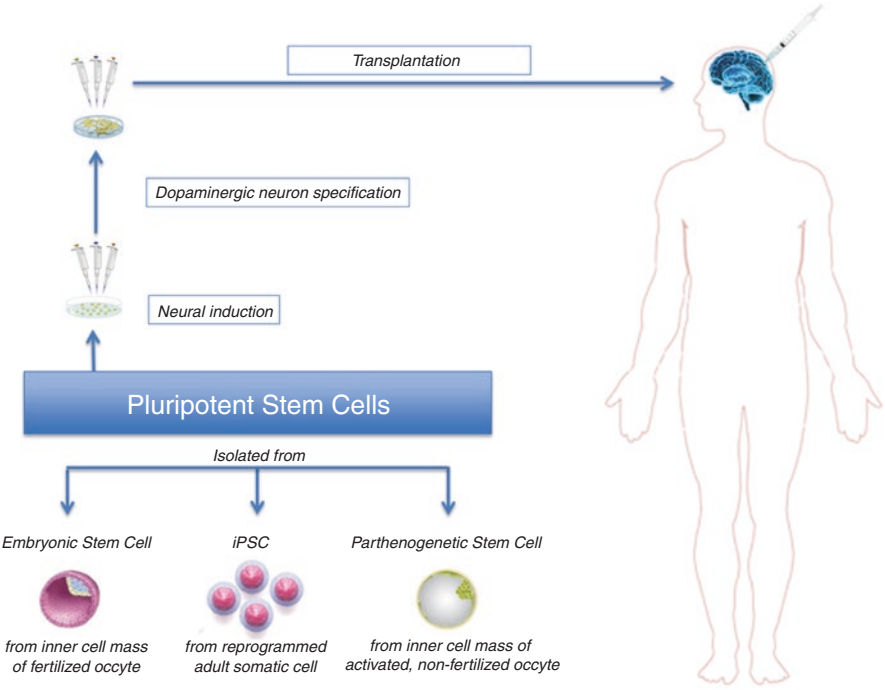

Fig. 5.3 Different types of human pluripotent stem cells available for CRT in PD after differentiation into Dopaminergic neurons

5.5 Conclusions and Future Directions

Conventional pharmacological treatments for most neurological disorders, including PD, only provide some symptomatic improvement but do not stop the progression of the degeneration. Therefore there is a clear need for alternative therapeutic strategies. The development of cell-replacement therapies using stem cells can provide substantial benefits for PD patients, as shown after transplantation of fetal cellular suspensions of dopaminergic precursors.

However, clinical studies to date have shown that there are still many gaps in terms of safety, effectiveness and overall methodology used in these implants. Among the aspects that need improvement, would be: (i) the development and standardization of surgical methods for cell transplantation and biological assays to evaluate the survival and effectiveness of the grafts. (ii) A good understanding of the effects of inflammatory and immunological processes on the progression of PD and on the implanted neurons. (iii) A better design and planning of clinical trials in terms of patient selection, evaluation criteria and patient follow-up. (iv) Identification of the best type of cell to be used as a source of DAn and standardization of protocols for the optimal production of these cells.

In relation to this last point there has been enormous progress in recent years; especially with regard to the generation of fully functional DAn from hESCs and hiPSCs. This has been possible thanks to a better knowledge of the signaling molecules that regulate embryonic development of DAn.

The DAn derived from human pluripotent stem cells can be obtained in large quantities in culture and have been shown to be able to survive long term implantation in preclinical models of PD being functional in a similar way to that observed with human fetal VM neurons. The main advantage of hiPSCs over hESCs is that they can be obtained from somatic cells of the own patient, therefore, could prevent immune rejection.

The derivation of clinically safe hiPSCs and their subsequent differentiation into DAn *in vitro* could provide excellent tools to replace the degenerated neurons in PD patients.

Furthermore the increasing results obtained from experiments using MSCs is also beginning to provide potential treatment options for PD. Though more studies need to be completed to confirm the safety and efficacy of the use of trans-differentiated MSCs for CRT, significant strides have been made in the use of MSCs as a neuroprotective treatment option. These cells have been show to promote neural and glial cell survival, control angiogenesis, inhibit apoptosis, have immunomodulatory functions and show neuroprotective actions through the release of a number of different neurotrophic and survival-promoting growth factors. A few clinical trials are already underway, utilizing allogenic and autologous-derived stem cells intravenously administered to see the beneficial effects of MSCs in humans.

In short, the perspective of an unlimited source of cells, in combination with the promising preclinical results suggests that CRT technology can be very close to a realistic clinical application in PD.

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