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Cellular Therapies for Huntington's Disease

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1. Introduction

Huntington's Disease is an autosomal dominant neurodegenerative disorder with an incidence of 5 to 10 per 100,000 in the Caucasian community. The clinical symptoms of HD are chorea, parkinsonism, dystonia, intellectual impairment, emotional and psychiatric disturbances as well as dysphasia, dysarthria, rigidity and gait disturbances. The depression that is associated with HD is thought to be secondary to the motor abnormalities, given that it develops prior to the appearance of any other symptoms (Folstein, 1989). To date symptomatic treatments are the only available treatments for HD as there are no disease-modifying therapies.

The pathology of HD is characterised by a loss of medium spiny projection neurons in the head of the caudate and putamen of the striatum (Ross and Margolis, 2001) which form part of a complex circuitry comprising parallel feedback loops involving discrete areas of cortex and subcortical structures. As a result of the neuronal loss there is eventually significant atrophy of these structures, with a compensatory expansion of the lateral ventricles. With disease progression the overall brain weight decreases by 25-30%, which reflects additional atrophy of other brain areas such as the cerebral cortex. Gliosis is also seen in the pathology alongside the marked neuronal loss. Neuronal loss in the cortex is found to be layer specific with the greatest loss seen in layer VI and significant amounts of loss seen in layers III and V (Reddy et al., 1999, Ross and Margolis, 2001). The relatively focal loss of medium spiny GABAergic projection neurons in the striatum presents an opportunity to explore neural transplantation as a strategy for cell replacement and circuit reconstruction. For neural transplantation to be successful it is dependant on the cells surviving the transplantation procedure and being able to integrate into the host brain (striatum) and become physiologically active (Lindvall and Hagell, 2002).

2. Clinical trials for neural transplantation

Much of the ground breaking clinical research on neural transplants was done for Parkinson's Disease (PD), beginning in the late 1980s. These trials used primary human foetal mesencephalic tissue as the donor tissue and transplanted it into the host striatum, which is the normal target area of these cells. The mesencephalic tissue contains fate-committed dopaminergic neuroblasts, which have the capacity to differentiate into fully mature dopaminergic neurons following transplantation. For this to be successful certain criteria need to be adhered to, these include harvesting tissue between specific gestational

ages and the optimisation of tissue preparation methodologies. If one considers the PD trials in which these principles are taken into account and which use good longitudinal assessment, then results to date in the PD trials have demonstrated improvements in a range of motor skills and many, but not all, of the patients have been able to reduce or even eliminate their daily intake of L-dopa (Hagell et al., 2002, Mendez et al., 2008, Olanow et al., 1996). However, there is variability in the success of this approach, which may be a direct result of variations in transplant methodology as well as differences in patient selection criteria (Freed et al., 2001, Freeman et al., 2000, Kordower et al., 2008, Li et al., 2008, Lindvall et al., 1990). Some of these trials have also highlighted the possibility of dyskinetic side effects in a proportion of patients (Freed et al., 2001), and the reasons for these is currently a topic of active investigation (Hagell et al., 2002, Carta et al., 2010, Lane et al., 2009a, Lane et al., 2009b, Politis et al., 2011, Politis et al., 2010, Steece-Collier et al., 2009). Following several years of round table discussions about how to move forward, a new multicentre European trial has been initiated and it is expected that patients will begin to be transplanted in 2012. This trial has taken all of the new data and the experiences learned from the past to ensure that the best possible protocols are adhered to in all centres, with the aim that the patients receive the best possible tissue transplant.

Parallel clinical trials of neural transplantation in HD are at a much earlier stage than the PD trials and are currently underway in a small number of centres around the world (Bachoud-Levi et al., 2000a, Freeman et al., 2000, Hauser et al., 2002, Kopyov et al., 1998a, Kopyov et al., 1998b, Rosser et al., 2002, Reuter et al., 2008, Philpott et al., 1997, Cicchetti et al., 2009). The French trial, based in Créteil, was the first to provide efficacy data, based on systematic long-term evaluation of their patients. Three of the five patients, having received bilateral striatal implants, were reported to show substantial improvement over several years (Bachoud-Levi et al., 2006, Bachoud-Levi et al., 2000c). More recently there has been an expansion of the French trial to include other French-speaking regions in Europe and a total of 40 patients will eventually receive transplants and will undergo follow-up, although no efficacy data is available as yet. In another study in Florida, 6 of 7 patients appeared to show improvement but one declined significantly, so that the overall group changes were not significant (Hauser et al., 2002). One patient died after 18 months due to cardiovascular disease and post mortem analysis of this patient's brain showed surviving graft tissue that was not affected by the underlying disease progression, at least at this time point (Freeman et al., 2000). The graft tissue was positive for striatal markers such as acetylcholinesterase, calbindin, calretinin, dopamine and tyrosine hydroxylase. Moreover, there was no sign of immune rejection in the graft region (Freeman et al., 2000). In the same study 3 patients developed subdural haemorrhages and 2 required surgical drainage (Hauser et al., 2002). These events may have been related to the stage of disease, which was rather more advanced than for the patients in the French or UK studies, in that more advanced cases of HD tend to have more cerebral atrophy with an accompanying increased risk of intracranial bleeding peri-operatively. Small numbers of patients have received grafts in several other centres with reports of safety (Kopyov et al., 1998a, Rosser et al., 2002), and although efficacy studies are underway in these centres, systematic reports have not yet been published. More recently it has been reported following post mortem analysis 10 years after transplantation in the Florida study, that the grafted cells had themselves been subjected to the disease process (Cicchetti et al., 2009). This raises issues about the long-term viability of transplantation as a therapy for HD, however, as this is only a report of 3 patients caution has to be taken not to misrepresent the field as a whole.

The UK trial (Rosser et al., 2002) is currently on hold due to EU regulations on tissue handling for transplantation, which now requires that all tissue be treated under good manufacturing practice (GMP). The French trial is not limited by such regulations due to their use of tissue pieces grafts as apposed to the cell suspension grafts used in the UK. There is little clear evidence to date stating that one method is better than the other (Watts et al., 2000) however, from the data that is available there is a suggestion that tissue pieces induce a more intense immune response post transplantation (Cooper et al., 2009). It is the belief in the UK that cell suspensions allow for greater integration of the cells into the host brain.

The initial studies of cell transplantation in HD are providing accumulating evidence of the conditions for safety, and preliminary evidence for efficacy. However, the limited availability of foetal tissue and the difficulty in ensuring the high degree of standardisation and quality control when a continuous source of fresh donor tissue is required from elective surgical abortion limits the widespread use of neural transplantation as a practical therapy. It has recently been shown that foetal tissue obtained from medical terminations of pregnancy is a viable source of tissue for transplant studies (Kelly et al., 2011). The use of medically sourced tissue will circumvent some of the logistical issues that were envisaged using surgical tissue due to the limited supply. Despite this new source of fresh foetal tissue there is still ethical and legislative concerns about abortion and the large number of donors required to support each operation, that restrict the number of patients that can receive grafts to a few specialist centres in a restricted number of countries. These issues have stimulated the search for alternative sources of donor cells or tissue that circumvent the problems associated with primary foetal tissue collection.

3. Alternative cell sources

The ability to generate a large stable population of cells to circumvent the supply issue and also to allow regular characterisation to ensure stability of the quality and character of the tissue, without the need for separate characterisation of each and every collection as is the case with primary tissue transplants is the ideal characteristic of an alternative cell source. Also, tissue storage methods need to be refined and validated so that the cells can be delivered on demand, to advance optimal clinical management of the recipient, rather than the surgeon and patient being constrained to surgery around an erratic schedule of tissue availability. The trials using primary foetal tissue thus provide a 'proof of concept' of the cell transplantation strategy as the basis for developing a practical therapy using a standardised, quality-controlled source of cells available to any appropriately equipped neurosurgical facility on demand. Several options are now being investigated as potential sources of donor tissue.

Stem cells are a possible source of cells for neural transplantation in HD and have attracted much attention in the last decade. Stem cells undergo self-renewal by symmetric division and can also undergo asymmetric division to produce another stem cell and a more differentiated progeny (Morrison et al., 1997, Watt and Hogan, 2000). Some multipotential cells may persist into adulthood, either by remaining quiescent in specific regions of the CNS parenchyma or by continued self-renewal (Morrison et al., 1997). Such cells are now referred to as "tissue specific stem cells" (Fuchs and Segre, 2000, Watt and Hogan, 2000). Embryonic stem (ES) cells have the potential to differentiate into all cell types under the

correct conditions. Stem cells from a range of sources have potential as donor cells for neural transplantation. However, whatever the source, therapeutic application will require that cells can be directed to differentiate into the precise phenotype required to replace the cells lost to the disease process, and specifically medium spiny neurons for HD. We describe here stem cell sources under consideration as potential donor cells in this context, and the extent to which directed differentiation has been achieved. This list is not exhaustive but covers at least the main categories of stem cells that are currently being explored as alternative cell sources for neural transplantation in HD as well as a number of other neurodegenerative disorders.

3.1 Adult neural stem cells (ANSCs)

ANSCs are tissue-specific stem cell and are derived from the mature brain. Altman and colleagues provided the first clear evidence, using 3H-thymidine autoradiography, that a low level of neurogenesis is ongoing in the dentate gyrus of adult rats (Altman and Das, 1965). ANSCs have since been confirmed in two main regions of the CNS: the sub granular layers of the dentate gyrus, from where the newly-formed neurons repopulate the dentate gyrus (Gage et al., 1995); and the subventricular zone (SVZ) of the lateral ventricles (Alvarez-Buylla et al., 2002), from where the newly formed neurons migrate via the rostral migratory stream to the olfactory bulb (Lois and Alvarez-Buylla, 1994). It has also been reported that neural stem cells may also reside in other regions of the brain, albeit at an even lower concentration, including cortex (Gould et al., 1999, Rietze et al., 2000) and the medial-rostral part of the substantia nigra pars compacta in the lining of the cerebroventricular system of the midbrain (Zhao et al., 2003), although these reports remain controversial (Frielingsdorf et al., 2004).

The attraction of ANSCs as a donor supply for neural transplantation would be the possibility of autologous transplants, thus bypassing the immunological issues of graft rejection, which can be severe in the case of xenografts and not entirely benign even for allografts. Furthermore, it may eventually be possible to recruit such cells for endogenous repair without a requirement for their isolation and re-implantation. That is, it might be possible to stimulate the resident population of ANSCs to migrate to the site of degeneration. However, adult neural stem cells remain difficult to isolate and grow in culture and the factors that would be required to enhance the proliferation of these cells and their differentiation into the particular phenotypes relevant to the site of degeneration remains unknown. Therefore, these cells are less likely to be of beneficial clinical use for transplantation in Huntington's Disease patients.

3.2 Neural stem (NS) cells

Neural stem (NS) cells are those cells that are derived from the developing or adult brain but which are already committed to a neural fate. These cells can be expanded in culture where they undergo asymmetrical division and have been shown to have the potential to differentiate into all cell types of the nervous system, neurons, astrocytes and oligodendrocytes. NS cells can be isolated from the developing brain and in the presence of specific growth factors such as EGF and FGF-2 these cells will form free floating spheres of cells "neurospheres". Animal experiments using these cells have been carried out using tissue from E14 (embryonic day14) mouse striatal tissue and it was verified that the spheres

formed from these cells are multipotential (Reynolds and Weiss, 1996). Clonal analysis in the presence of FGF-2 has shown it to be mitogenic for NS cells (Drago et al., 1991, Gensburger et al., 1987, Ray and Gage, 1994, Ray et al., 1993, Richards et al., 1992, Vicario-Abejon et al., 1995).

Several growth factors have the potential to enhance the neuronal differentiation of these cells down particular lineages, including nerve growth factor (NGF), insulin-like growth factor (IGF) and tumour necrosis factor (TNFα) (Arsenijevic et al., 2001, Cattaneo and McKay, 1990, Santa-Olla and Covarrubias, 1995, Tropepe et al., 1997). Identifying an appropriate growth factor cocktail appropriate to the phenotype associated with each particular application may be a necessary prelude to using these cells for transplantation.

It has been found with molecular characterisation of foetal NS cells in vitro that they retain a degree of their site-specific identity when environmental cues are absent but when co-cultured with cells of different origin they can adopt a new fate (Fricker et al., 1999, Parmar et al., 2002). IsletI and Er81 are genes associated with striatal development and their expression is found to be maintained over time in culture, but with neuronal differentiation, expression of striatal specific neuronal markers such as DARPP-32 and Islet1 are lost, although they do express homeobox transcription factors Dlx and MEIS2, which are associated with ventral forebrain development (Parmar et al., 2002, Skogh et al., 2003). Thus, it appears that expansion of NS cells in culture may restrict the differentiation potential of the cells. Further evidence for this has been demonstrated in disease models where these NS cells can survive post-transplantation following a short period of expansion; but that this is compromised by longer expansion times (Zietlow et al., 2005). One interpretation of these findings is that positional information is lost with continued expansion so that when long-term expanded cells are placed in an environment such as the adult CNS, they are not exposed to the developmental signals that they would see in the developing brain and are thus unable to differentiate into neurons appropriate to the site from which they were derived (for example medium spiny neurons from striatally-derived NS cells). However, when grafted to the neonatal brain, similar cells appear to respond to developmental signals and regional determinants by differentiating in a site-specific manner (Englund et al., 2002a, Englund et al., 2002b, Rosser et al., 2000) suggesting that they retain the capacity to respond to developmental signals if they are present.

From these and many other studies it is clear that NS cells may have the potential for neural transplantation. However, for this to become successful it is imperative that we first optimise the conditions in which these cells are expanded so as to increase the frequency at which these cells differentiate into the appropriate phenotype. It may be that we are now at a point where we can take what has been learnt from the directed differentiation of stem cells (as described below) and developmental biology and apply it to NS cells. Such factors as sonic hedgehog and dikkopf to mention but a few, which have been identified as important in striatal development and for directing the differentiation of stem cells, may be of benefit to maintaining the positional identity of the NS cells as well as directing the undifferentiated cells within this culture system that have not yet gone through their terminal differentiation, given the heterogeneic nature of the cells in question (El-Akabawy et al., 2011). The immunogenicity of these cells is another factor that needs to be taken into consideration for neural transplantation. Several studies have looked at the immunogenicity of these cells (Akesson et al., 2009, Al Nimer et al., 2004, Hori et al., 2007, Odeberg et al.,

2005, Ubiali et al., 2007, Laguna Goya et al., 2011) and it has been shown that these cells when expanded in culture become more immunogenic, and so patients receiving these cells would need to be immunosuppressed to prevent graft rejection taking place.

3.3 ES cells

Embryonic stem (ES) cells are generated from the inner cell mass (ICM) of the blastocyst passing the first step of cell differentiation and giving rise to trophectoderm (Evans and Kaufman, 1981, Martin, 1981). ES cells have the ability to extensively proliferate and self-renew whilst maintaining their pluripotentcy. ES cells are able to differentiate into all cell types of the three germ layers—ectoderm, mesoderm, and endoderm, and when transplanted are capable of germline transmission to generate chimeric animals which is in contrast to embryonic carcinoma cells (EC) cells (Bradley et al., 1984). Thus, ES cells can be used to generate models of disease and to understand developmental pathways by introducing modifications into the mouse germline. Human ES (hES) cells were first derived in 1998 (Thomson et al., 1998) and also have the potential to differentiate into cells of all three germ layers (Amit et al., 2000). However, the conditions used for culturing mouse and hES cells are different in that hES cells do not survive in leukemia inhibitory factor (LIF) containing media, a prerequisite for mouse ES cell culture (Daheron et al, 2004; Humphrey et al., 2004). They are pluripotent and can be propagated in culture for long periods of time in an undifferentiated state (Blau et al., 2001, Odorico et al., 2001, Schuldiner et al., 2001).

The ability of these cells to divide in culture over long periods of time highlights their potential for cell transplantation in that they would alleviate the logistical issues associated with primary tissue transplants. However, despite this, the important step of directing the differentiation of these cells into specific cell types is proving difficult. Whilst the default differentiation pathway appears to be that of a neural lineage, the specificity of the neuronal differentiation is limited. In the Parkinson's Disease model where large numbers of dopamine (DA) neurons are required there has been significant developments in directing the differentiation of ES cells into the required phenotype using factors such as retinoic acid and sonic hedghog (shh). In the case of HD where a population of medium spiny DARPP-32 positive neurons are required there is a very limited literature. Bouton and Kato (Bouhon et al., 2006, Kato et al., 2004) have reported on their ability to direct the differentiation of mouse ES cells into a ventral lineage where the cells maintained the expression of ventral markers that are typical of striatal neurons over a short time in culture. In the case of human ES cells, to date there is only one report of DARPP-32 positive neurons (Aubry et al., 2008). In this study the authors report that the DARPP-32 positive cells generated in culture had the ability to differentiate into such mature neurons in vivo following xenotransplantation into the lesioned rat brain model of HD. However, this is the only study reporting such data and to date there is no further evidence of a working protocol generating large populations of these neurons from stem cells. As part of a European consortium to address this issue several groups have come together to facilitate this issue and it is anticipated that new data will come to the fore in the not too distant future.

There have been significant ethical disputes associated with the derivation and use of human ES cells, including concerns over the use of human embryos, and fears related to

their potential for human cloning (McHugh, 2004, Sandel, 2004). As a result of these ethical issues many countries have restricted or banned human ES cell research. Nevertheless, other countries have actively supported the development of human ES cell research because of the perceived potential for therapeutic benefit in a wide range of diseases. Some, including the UK, allow cloning of human embryos for therapeutic purposes, while imposing tight regulations to preclude their use for reproductive cloning.

However, despite the great potential of these cells one major caveat is their potential to form tumours. It is crucial that a method be developed for eliminating any possible ES cells from the differentiated population as one single ES cell could have devastating affects if transplanted into the host brain.

3.4 Induced pluripotent stem (iPS) cells

Induced pluripotent stem cells were first described in 2006 (Takahashi and Yamanaka, 2006). Introducing four exogenous transcription factors to differentiated cells and nurturing those cells in an embryonic environment the authors were able to directly reprogramme the cells as pluripotent like cells. Yamanaka and Takahashi in 2006 after much work and many different combinations of factors concluded that the four factors- Oct4, Sox2, c-Myc, and Klf4, which are present at high levels in ES cells are sufficient to transform mouse fibroblasts into cells that mimic ES cells (Figure 1). Subsequently, there have been many reports strengthening this finding (Brambrink et al., 2008, Kaji et al., 2009, Maherali et al., 2007, Meissner et al., 2007, Nakagawa et al., 2008, Stadtfeld et al., 2008, Takahashi et al., 2007, Wernig et al., 2007, Yamanaka, 2008, Yu et al., 2007, Okita et al., 2007, Wernig et al., 2008). iPS cells morphologically look identical to ES cells and as a result more detailed epigenetic characterisation is required to confirm the presence of iPS cell colonies in culture (Figure 2).

Following from this initial work, there have been many refinements to the initial viral based protocol using various methods from a non viral approach to small molecules to generate similar cells and using different factors (Haase et al., 2009, Kim et al., 2009, Sidhu, 2011, Soldner et al., 2009, Yu et al., 2009, Kaji et al., 2009, Okita et al., 2008, Park et al., 2008b, Stadtfeld et al., 2008, Woltjen et al., 2009) with each reporting positive generation of iPS cells with different levels of efficiency. In 2008 the first human iPS cells were described (Park et al., 2008a, Park et al., 2008b) and as with mouse iPS cells there has since been several reports of iPS cell generation from human tissues including; human fetal tissue, adult neural cells, adult fibroblasts, foreskin and disease specific sources, thus highlighting the potential usefulness of these cells both from a scientific and clinical perspective.

The molecular mechanisms of somatic cells reprogramming are still unclear. The wide range of time points to recognise the iPS colonies were reported from numerous studies; the epigenetic events during the reprogramming process and the up or down regulation of involving pluripotent genes are still a mystery. It is not known whether the reprogramming of somatic cells to pluripotent cells is a timed sequential process or if it's a random process, but what is important is that more studies are needed in order to understand the molecular function and use these cells before they are to be used in any clinical research.

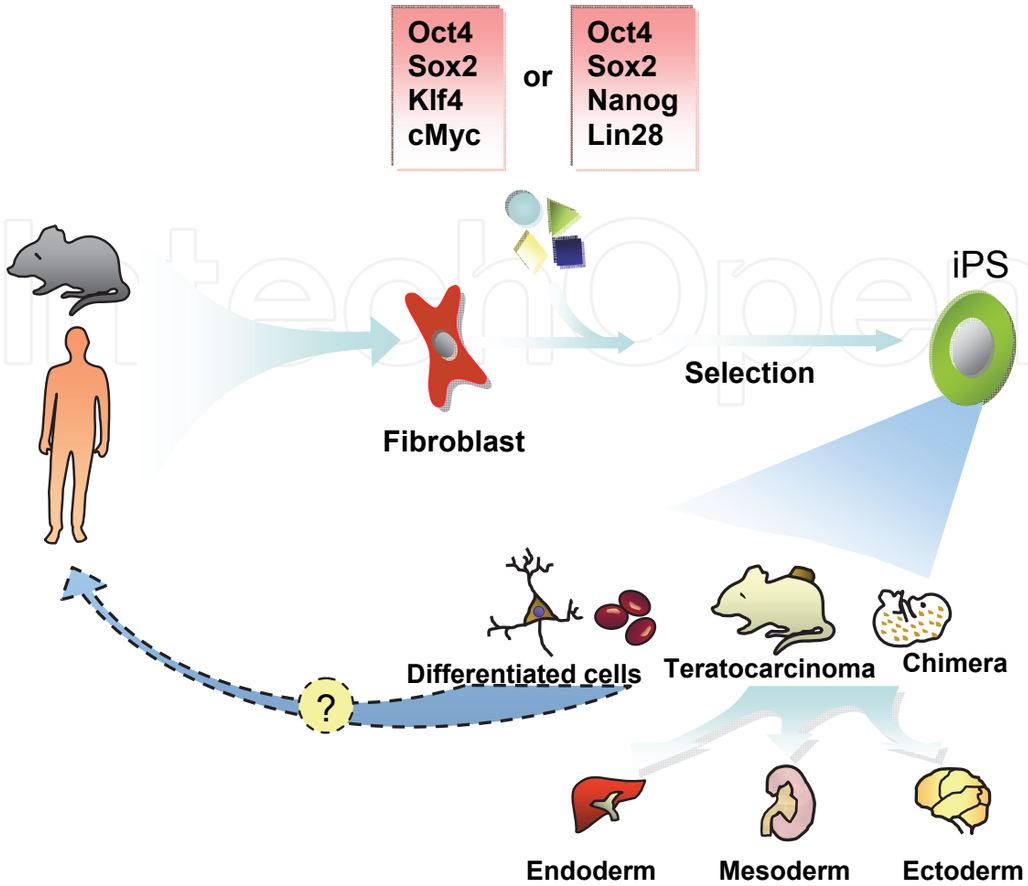

Fig. 1. Schematic drawing demonstrates direct reprogramming mouse and human fibroblasts to iPS cells with four defined factors Oct4, Sox2, Klf4, c-Myc, and Lin28. After selection, iPS cells are derived. These cells could differentiate into all cell types within three germ layers as assayed by teratoma formation and developmental contribution. (Adapted from (Welstead et al., 2008)

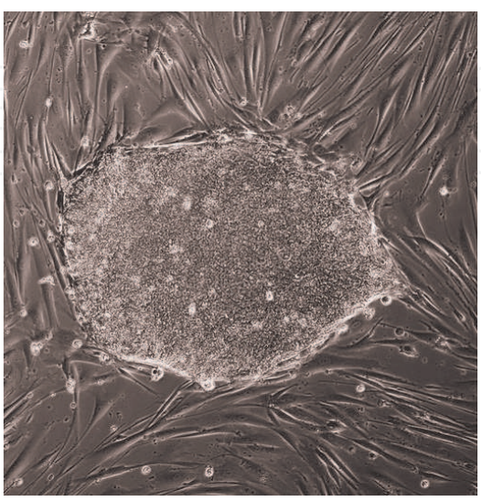

Fig. 2. A colony of pluripotent stem cells derived under non viral conditions.

3.5 Therapeutic potential of iPS cells

The ultimate goal of iPS cell derivation for degenerative diseases is to provide transplantable patient-specific cell sources that overcome ethical and immune rejection difficulties as found with other donor cell sources. Hematopoietic progenitors (HPs) derived from iPS cells were the first to be reported to successfully rescue and improve all pathological conditions in a mouse sickle cell anemia model (Hanna et al., 2007). This early study is a promising step forward in the quest to generate a suitable cell source for transplantation studies.

Interestingly, one year later, Wernig reported the functional benefits and behavioral improvements using iPS cell derived NSCs transplanted into a PD rat model (Wernig et al., 2008). Wernig claimed that in vitro NSCs generated from an iPS cell line were able to differentiate into all neural cell types (β-III tubulin, GFAP, O4 positive staining) including dopaminergic neurons after patterning factors had been added to the culture (FGF8 and sonic hedgehog) (Wernig et al., 2008). In addition, migration of NSCs from iPS cells was observed in various brain regions such as the striatum, midbrain, and hypothalamus after injection to the lateral ventricle of mouse embryos (E13.5-14.5). Furthermore, functional restoration and behavioral improvement were confirmed by action potential initiation, synaptophysin expression, and bias movement reduction from reprogramming somatic cells derived neurons (Wernig et al., 2008).

These studies provide evidence that iPS cells have high potential as an alternative donor cell source for cell replacement in regenerative medicine. To date there is no literature pertaining to the differentiation of DARPP-32 positive medium spiny neurons from iPS cells however, given the fast pace of this ever evolving field it is anticipated that such data will become available in the not too distant future.

However, as with ES cells and NSCs there is an associated risk of tumour formation. In comparison to the other cell sources, iPS cells have the potential to be generated on a patient specific basis and so may overcome this issue to some extent. The need to go through the ES like state is problematic as it renders the cells tumorigenic in nature and so makes them less attractive than initially envisaged.

Recently there have been reports about the possibility of generating functional neurons from fibroblast cells without having to go through the stem cell state (Parmar and Jakobsson, 2011, Pfisterer et al., 2011). As with iPS cells this is a viral based protocol using a combination of three different factors, namely; Ascl1, Brn2 and Myt1l (Forsberg et al., 2010, Ieda et al., 2010). This exciting development circumvents the issue of tumorgenicity that is associated with current ES and iPS protocols but is limited in that large numbers of cells cannot be generated. Therefore whilst this approach currently is optimistic, there are issues that will need to be overcome if this is to become a viable option for cell therapy.

3.6 Trans-differentiation of other tissue-specific stem cell populations

Another approach is to attain trans-differentiation of a non-neural tissue-specific stem cell population, the classic one being bone-marrow-derived stem cells. This population have the advantage of being more easily harvested than either foetal or adult neural stem cells, but the disadvantage that they do not by default produce neurally differentiated cells.

There is some evidence that trans-differentiation can be achieved, although this remains an area of dispute. Mesenchymal stem cells (MSCs) which are derived from the bone marrow and under normal conditions give rise to chondrocytes, adipocytes and cells of the blood lineage have been reported to trans-differentiate to ectodermal and endodermal cell fates (Zhao et al., 2002). In vitro, MSCs have also been shown to differentiate to form neurons and astrocytes. MSCs transplanted into the rat brain survive and express markers of neuroectodermal cells as well as having a functional effect (Deng et al., 2006, Bertani et al., 2005). This ability to trans-differentiate is not unique to MSCs, as neural stem cells have also been shown to have this ability, where they were seen to differentiate into muscle (Galli et al., 2000). However, evidence suggests that this plasticity may be a result of cell fusion based on studies that have looked at the potential of MSCs to differentiate into hepatocytes (Vassilopoulos et al., 2003, Wang et al., 2003), the increasing body of evidence for MSC trans-differentiation would suggest that this may in fact be true. This issue will need to be clarified for these cells to be serious contenders for neural transplantation.

The best characterised of the tissue-specific stem cells are the Hematopoietic stem cells (HSCs), which are also derived from the bone marrow, and reconstitute the blood. Two classes of HSC have been identified in mouse, those that survive for around 2 months, (the short term, ST-HSC), and those that survive for greater than 6 months, (the long term, LT-HSC) (Blau et al., 2001). Fluorescence-activated cell sorting (FACS) has been used to positively select cells based on the expression of specific cell surface markers. HSCs can be highly enriched up to 10,000 fold and then transplanted into the bone marrow of patients (Lagasse et al., 2001) for the treatment of oncogenic blood diseases. In an animal model of spinal cord injury, HSCs have been shown to survive for 5 weeks after transplantation, differentiate into astrocytes, oligodendrocytes and neuronal precursors and show improvement in functional behaviour using hindlimb motor function (Koshizuka et al., 2004), although no mature neurons were identified.

Human umbilical cord blood is easily retrieved following labour without the risk of harm to the mother or child and has been reported to contain multipotential progenitor cells that apparently have the ability to trans-differentiate into neuronal and glial cells (Sanchez-Ramos et al., 2001). Transplantation of these cells into the neonatal and adult brain have shown potential to survive and differentiate into neurons and glia (Li et al., 2004, Nan et al., 2005, Sanberg et al., 2005, Willing et al., 2003, Zigova et al., 2002). It may be that intravenous delivery rather than neural transplantation will be a more advantageous method of administering these cells for therapeutic benefit, based on a study by Willing et al (2003) where there was significant improvement in certain behavioural tasks when compared to animals receiving neural transplants of cells directly to the striatum. However, further studies are necessary to validate the potential of these cells and again, the issue of cell fusion needs to be addressed in this context.

3.7 Xenogenic tissue

Xenotransplantation offers the opportunity of breeding animals for foetal striatal tissue donation under conditions where the supply can be regulated according to demand; where the breeding stock is inbred, well characterised and controlled for pathogens; and where tissue collection and preparation can be undertaken under standardised sterile good

manufacturing practice (GMP) conditions. The most likely donor candidate is porcine tissue, the advantages being: the extensive experience of animal husbandry within this farm species; the reliability of breeding; the large size of the litters; the possibility of sterile collection under standardised conditions; the comparable size and time course of development of the pig and human brain; and the potential application of transgenic technology to porcine tissue, which would open up the possibility of genetic manipulation, for example to modify the immunogenicity of transplanted tissue.

Transplantation of xenogeneic tissues into the immunosuppressed host CNS has been performed using a number of species, for example human to rat, pig to rat, rat to mouse and vice versa (Armstrong et al., 2002, Deacon et al., 1999, Galpern et al., 1996, Garcia et al., 1995, Isacson et al., 2001, Svendsen et al., 1997). Both primary and expanded tissue graft experiments have been reported using xenogenic tissue. The grafted tissue has been found to survive transplantation, axonal and glial fibre projections from the grafts, and make synapses with the host brain.

Clinical studies of CNS xenotransplantation are limited. Primary porcine embryonic striatal tissue has been transplanted into the caudate and putamen of 12 immunosuppressed PD patients with some clinical improvements reported, although there was little convincing evidence of graft survival (Isacson et al., 2001). The immune response from these grafts was more vigorous than that seen in human to rodent models. One patient died 7 months post-operatively for reasons unrelated to the graft, and was found to have very small numbers of surviving neurons in the graft region, raising the possibility that the majority had been rejected. In the same series, 12 HD patients received porcine striatal grafts but, again, there was little evidence of graft survival or functional effect. Twelve months of post-operative analysis of these patients demonstrated no change in the mean total functional capacity score (Fink et al., 2000).

Two key issues need to be resolved for xenografts to progress to practical therapeutic trials. The first relates to the fact, as illustrated by the first pilot clinical trial reported above, that xenografted tissue is largely rejected in the absence of effective immune protection. Two alternative strategies were adopted in the Diacrin trial – daily treatment with CsA or treatment with an antibody against major histocompatability complex 1 (MHC 1) to block the host T cell response (Fink et al., 2000). There is no clear evidence that either strategy proved effective for yielding good cell survival in patients, and it is surprising that the study had progressed on the basis that preliminary reports of the same strategies in primates were equally ineffective. Combination immunoprotection strategies to promote xenograft survival are an area of active research (Armstrong et al., 2001, Harrower and Barker, 2004). We described a new method that would allow long term xenograft survival of human fetal tissue in the rat brain (Kelly et al., 2009). In this method we took advantage of the naïve state of the neonatal rat pup immune system and induced what we describe as desensitisation to xenogenic tissue. Subsequent neural transplants into the adult rat brain resulted in good graft survival up to 40 weeks post transplantation without any immunosuppression. This method now allows long-term evaluation of xenogenic cells both anatomically and more importantly functionally in animal models of disease. Another approach that has been described to block the immune response to ES and iPS derived cells is to use an antibody response against the co-stimulatory molecules

involved in the T cell response (Pearl et al., 2011). This method has only been reported for short-term graft survival and as yet no long-term efficacy data is available for functional evaluation of xenogenic cells using this approach.

The second key issue that requires resolution relates to safety of xenografted tissues. In the light of the recent spread of bovine spongiform encephalopathy to man in the form of new variant Creutzfeld-Jacob Disease, and the difficulty in controlling the spread of animal pathogens, as exemplified by the recent UK foot-and-mouth epidemic, there is widespread concern world-wide about the difficulties of eliminating the possibility of transmitting animal diseases to man. This may be particularly risky in the context of transplantation of tissues directly into the immunosuppressed CNS. The concern is not just for the recipient but, in the case of porcine endogenous retrovirus (PERVs), whether direct transfer into the brain might provide a route of transmission that allows virus mutation into new forms of viruses that give rise to unpredicted new diseases in man, even giving rise to de novo epidemics. Although the chances of such mutation are recognised to be very low, the cost of occurrence could be devastatingly high. Moreover, the risk of generating a new disease by an unknown mechanism is one that it is impossible to absolutely exclude by any known safety screen. The regulatory climate is consequently such that any novel xenograft approach is unlikely to gain approval for trial in the foreseeable future, at least in Europe. In the absence of having suffered the same major BSE, CJD and FMD epidemics, US regulations, although strict, are somewhat more permissive, with the result that most academic and commercial research of developing xenotransplantation as a therapeutic strategy for the CNS has moved westwards across the Atlantic over the last 10 years.

3.8 Genetically engineered cells

A variety of cells may be engineered in vitro either for the purpose of producing molecules of potential importance for CNS release (for example, in the form of polymer encapsulated cells, as below), or to alter the properties of a cell to render it potentially useful for circuit reconstruction. Of course, these strategies are not necessarily mutually exclusive - trophic factor support may be crucial for transplanted cells to survive and integrate in the host brain, and genetically engineering cells to release trophic factors in the graft region is one potential method for optimising graft survival.

The herpes simplex viral vector was the first virus to be tested as a method of introducing genes into the adult CNS (During et al., 1994, Fraefel et al., 1996, Song et al., 1997). More recently, other viral vectors have been introduced, including adenovirus, the recombinant adeno-associated virus (rAAV), lentivirus and pseudotyped vectors. The rAAV vector is more efficient than the HSV in that it is possible to achieve much higher levels of expression. The use of such vectors has allowed genes to be transferred to a specific group of cells in the CNS (Janson et al., 2001), and has provided support for the efficacy of factors such as GDNF for PD (Eslamboli et al., 2003, Kirik et al., 2000, Mandel et al., 1999, Mandel et al., 1997) and CNTF for HD (Emerich, 2004, Kahn et al., 1996, Mittoux et al., 2002, Regulier et al., 2002).

Polymer capsules have been considered as a system for trophic factor delivery to the CNS as they have the advantages of being relatively cheap to produce and can also be removed from the CNS as required, but the major drawback is that the effect is not long lasting

(Emerich et al., 1994). Where a limited amount of a protein is required for relatively short periods of time, polymer microspheres are an attractive alternative as they are biodegradable and subsequent surgical procedures are not required for retrieval (Date et al., 2001). However, improvements in the duration of release have been obtained by the use of encapsulated cells engineered to produce the desired molecules (Emerich, 1999, Emerich et al., 1997). Here, cells engineered to secrete specific substances such as neurotrophic factors are protected from the host immune system by a semi-permeable selective biocompatible outer membrane (Emerich, 1999, Emerich, 2004, Emerich et al., 1998, Emerich et al., 1997, Emerich et al., 1996). The outer membrane allows the entry of nutrients to the cells whilst also allowing the exit of neuroactive molecules. The advantage of this strategy is that it allows for the implantation of xenogeneic cells, which may be much easier to obtain or engineer than human cells. This approach has been used for delivery of factors such as GDNF in animal models of PD (Date et al., 2001, Sautter et al., 1998) and CNTF in animal models of HD (Emerich et al., 1997).

In the case of HD there have been several studies using polymer encapsulated cells for the delivery of CNTF. Baby hamster fibroblasts have been genetically modified to produce hCNTF and incorporated into polymer capsules (Anderson et al., 1996, Emerich et al., 1996). Both rodent and primate studies have been carried out incorporating this method (Anderson et al., 1996; Emerich et al., 1996; Emerich et al., 1997; Emerich and Winn, 2004; Kordower et al., 2000; Mittoux et al., 2000). These animal studies suggested that CNTF can protect striatal neurons against subsequent damage from an excitotoxic lesion. As well as protecting specific populations of striatal neurons from lesion-induced cell death, behavioural improvement was observed on skilled motor and cognitive tasks when compared to control animals. Encapsulated CNTF released by BHK cells were used in a clinical trial in France (Bachoud-Levi et al., 2000b), however, it was subsequently found that on removal of the capsule the cells failed to release sufficient amount of CNTF. The trial has reported safety and feasibility of this approach but further work is required to optimise the capsule for release of CNTF (Bloch et al., 2004). Nevertheless, the use of encapsulated cells for the delivery of growth factors and neurotrophic factors is an attractive alternative and may be required in combination with neural transplantation as a means of providing trophic support to the grafted cells.

Another potential cell source is immortalised cell lines, the neurally committed lines, such as the Ntera2 cell line, RN33B and Hib5. Functional benefit has been reported using these cells in various animal models (Catapano et al., 1999, Lundberg et al., 1996, Miyazono et al., 1995, Saporta et al., 2001). The Ntera2 cell line has been the most widely used. These cells are derived from human embryonal carcinomas and are terminally differentiated in vitro with retinoic acid. They have been found to respond to environmental cues when transplanted into the excitotoxically lesioned striatum (Saporta et al., 2001; Miyazono et al., 1995), sending out target-specific projections as well as expressing a site-specific phenotype. Grafting Ntera2 cells into the excitotoxic lesioned striatum resulted in neuronal differentiation, and a preliminary study reported rather dramatic functional effects (Hurlbert et al., 1999). However, on more detailed analysis the cells did not express any striatal-specific markers and there was no sustained improvement on skilled paw reaching and cylinder placing (Fricker-Gates et al., 2004). Transplantation of the RN33B cell line to the lesioned and non-lesioned striatum of rats has demonstrated their potential to differentiate into neurons in a

site-specific way and form connections with target areas such as the globus pallidus (Lundberg et al., 1996), although only a proportion of the cells showed this differentiation potential. A major disadvantage of using such cell lines is the genotypic variability that arises from the immortalization process (Renfranz et al., 1991), and the risk that cells continue to proliferate to form tumours after transplantation.

4. Good manufacturing practice (GMP)

One of the major stumbling blocks for clinical trials of cell transplantation in Europe has been the introduction of the human tissue directive. This directive stipulates that all tissues used for human patients must be handled under clean room conditions. As a result many trials have been stopped to allow time to implement these new conditions. Each member state of the EU has taken their own interpretation of the directive and in the UK this is regulated by the human tissue authority (HTA). It is a requirement that any facility used to manipulate the cells/tissue used for transplantation into patients be licenced and governed by a strict set of guidelines. As a result in the case of the UK trial (NEST-UK) no patients have been transplanted in the last decade.

5. Conclusion

For HD, Cell transplantation is a promising therapy based on the current data from clinical trials. However, it is limited by the availability of a reliable source of cells that can replace the lost cells and reform the connections required for functional benefit. The proof of principle data from human fetal tissue studies highlights the effectiveness of the approach and the need for an alternative cell source. This chapter has highlighted some of the possible alternatives available and the potential of each one. Whilst the focus is very much on stem cells it is important that other cells also be considered as each has its own caveats. Which cell source is likely to make it to the clinical is not certain at this time and it is clear that much work is required before this can happen. Whilst much is known about cell sources and their potential for PD it is clear that the HD field is a long way behind. It is important that we as scientist stay focused on the goal and work together to move the field forward. As well as the issues of differentiation, tumour formation and cell number we also need to be mindful of the regulatory issues when devising such protocols and how they will adapt to GMP conditions. There is increasing emphasis on the use of GMP grade products for such studies, which are widely available. HD is a devastating disease and we are driven by the need for a therapy that works to make the lives of these patients less distressful.

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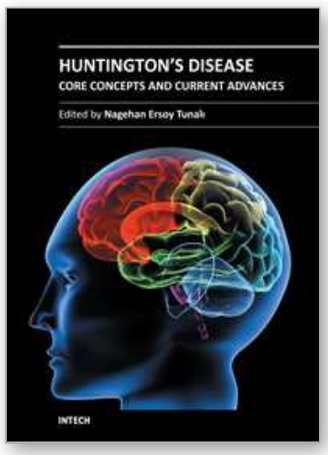

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Huntington's Disease is one of the well-studied neurodegenerative conditions, a quite devastating and currently incurable one. It is a brain disorder that causes certain types of neurons to become damaged, causing various parts of the brain to deteriorate and lose their function. This results in uncontrolled movements, loss of intellectual capabilities and behavioural disturbances. Since the identification of the causative mutation, there have been many significant developments in understanding the cellular and molecular perturbations. This book, "Huntington's Disease - Core Concepts and Current Advances", was prepared to serve as a source of up-to-date information on a wide range of issues involved in Huntington's Disease. It will help the clinicians, health care providers, researchers, graduate students and life science readers to increase their understanding of the clinical correlates, genetic aspects, neuropathological findings, cellular and molecular events and potential therapeutic interventions involved in HD. The book not only serves reviewed fundamental information on the disease but also presents original research in several disciplines, which collectively provide comprehensive description of the key issues in the area.

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