

Producing striatal phenotypes for transplantation in Huntington's disease

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Abstract

Neural transplantation as a therapeutic strategy in neurodegenerative disorders offers to replace cells lost during the disease process, with the potential to reconstruct dysfunctional circuitry, thus alleviating associated disease symptoms. The focal loss of striatal cells, specifically medium-sized spiny neurons (MSN) in Huntington's disease (HD), makes transplantation a therapeutic option. Here, we review the progress made in generating striatal MSN phenotypes for transplantation in HD. We discuss the use of primary fetal tissue as a donor source in both preclinical and clinical studies and assess the options for renewable cell sources. We evaluate progress in directing the differentiation of renewable cells towards a striatal MSN phenotype for HD.

Keywords: Huntington's disease, medium-sized spiny neurons, neural transplantation, primary fetal tissue, embryonic stem cells

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Introduction

Huntington's disease (HD) is an autosomal dominant disorder characterized, at least in the early stages, by extensive dysfunction and atrophy of striatal projection neurons, specifically the medium-sized spiny neurons (MSNs).^{1,2} The dysfunction and severe atrophy of the striatum causes disruption to the basal ganglia circuitry, and this is largely responsible for the symptoms associated with HD, including motor, cognitive and psychiatric impairments.³ In the absence of a disease-modifying therapy for HD, only symptomatic treatments are available which partially treat a subset of symptoms. The specificity of cell loss and the focal site of this loss in HD makes transplantation a viable therapeutic option. Neural transplantation aims to replace the neurons that are lost in the disease process. The expectation is that transplanted cells would develop new connections within the host brain, enabling reconstruction of the circuitry, which in turn would alleviate symptoms associated with the disease and bring about functional improvements.⁴

Primary fetal donor tissue

Primary fetal striatal tissue offers a source of donor MSN precursors and is currently the 'gold standard' for

transplantation in HD.⁴ Cells harvested from the putative embryonic striatum (termed the whole ganglionic eminence: WGE) have been exposed to temporal and spatial developmental cues normally present in that region, and thus provide a source of 'pre-programmed' MSN precursors that have the capacity to generate properly specified striatal MSNs. Studies using primary tissue are important in order for us to understand how to develop protocols for full specification of MSNs from alternative sources of donor cells. One approach to a better understanding of the nature of mature MSNs is to characterize them throughout development in order to identify markers of these cells and to understand in detail the regulatory processes necessary for generation of properly specified MSNs.

The most commonly used HD host for transplantation studies is the quinolinic acid (QA) striatal lesion model (QA lesion model). QA is toxic to gamma-aminobutyric acid (GABA)-ergic MSNs while sparing the cholinergic and aspiny interneurons.⁵ Other animal models, such as transgenic HD mice,^{6,7} that might resemble the human condition more closely are also available, although, to date, are less well-characterized for transplantation purposes.

Preclinical studies, using rodent tissue, have investigated the optimum parameters for transplantation into the HD-like degenerated brain, such as age of the developing embryo, region of the developing brain for dissection and

tissue preparation.^{8–10} The embryonic donor age of the tissue is critical to the success of the transplant. A study by Fricker *et al.*⁹ used dopamine and cyclic adenosine 3',5'-monophosphate-regulated phosphoprotein, 32 kDa (DARPP-32) and acetylcholinesterase (AChE) immunohistochemistry to determine the presence of striatal-like tissue, and reported that WGE cells derived from embryonic day (E)14 and E16 donors were richer in striatal cells than those derived from older embryos. However, functional recovery of animal recipients of these grafts, as measured using a range of behavioral tests, was only improved using E14 donors and was not achieved with E16 cells.¹⁰ This emphasizes the importance of using functional assessments in preclinical studies rather than relying on anatomical analysis alone. The optimum gestational window for human donor fetal striatum has not yet been determined. However, transplantation of human primary WGE into rodent models of HD suggests that the window may fall between 6 and 10 weeks gestation (postconception),^{11–13} although further refinement through systematic studies is required.

Although it is clear that a functional graft for HD requires donor cells from the developing striatum (WGE), this region is heterogeneous in terms of cell composition and gene expression.¹⁴ The WGE may be divided into lateral (LGE) and medial (MGE) parts: the former giving rise predominantly to MSNs and the latter predominantly to interneurons.^{15,16} Fate mapping studies revealed that cells originating in the LGE migrate both anteriorly and ventrally, giving rise to projection neurons of the striatum, nucleus accumbens and olfactory tubercle, while the majority of cells originating in the MGE migrate dorsally and generate interneurons expressing GABA, parvalbumin and somatostatin.¹⁷ Considering this, it is not surprising that striatal grafts, comprised of WGE or regional dissections, are heterogeneous with respect to neuronal phenotype containing regions with striatal-like tissue, as well as neurons characteristic of adjacent regions such as the cortex and globus pallidus.^{8,18} Donor cells derived purely from the LGE generate grafts that contain a greater proportion of DARPP-32- and AChE-immunopositive cells compared with WGE-derived grafts, and a markedly higher proportion than MGE-derived grafts,^{19,20} as would be expected considering their cellular potential as described above. With respect to functional recovery, LGE-derived grafts resulted in significantly reduced drug-induced rotations, with no such effect seen with the other regional dissections.²⁰ However, significant graft-mediated amelioration of paw reaching deficits was only seen in MGE-derived grafts, and not those derived from LGE or WGE.²⁰ Despite the fact that LGE alone results in grafts with greater striatal-like tissue, these observations support an inclusive dissection of WGE to allow for superior functional recovery following striatal transplantation.

Human fetal primary donor cells for transplantation into patients with HD are currently obtained from elective termination of pregnancy under full ethical consent from the maternal donors.^{21,13} Upon retrieval of fetal tissue, the developing striatum is dissected and tissue may be stored in hibernation medium for up to a week before

transplantation, without a reduction in viability.^{22,23} Previously, fetal tissue was retrieved solely from surgical terminations of pregnancy (STOP). However, we have recently provided evidence which supports the use of fetal tissue retrieved from medical terminations of pregnancy (MTOP), identifying this method as a viable source of cells for neural transplantation.¹³ Due to the less traumatic nature of the procedure, tissue is markedly less fragmented when retrieved from MTOP rather than from STOP, which subsequently results in a more reliable and accurate dissection.¹³

Clinical trials for cell-replacement therapy in HD using fetal tissue are ongoing and have demonstrated proof-of-principle that neural transplantation can be beneficial in HD, but consistent efficacy has not yet been achieved. Human fetal striatal grafts in symptomatic HD patients can survive and show typical morphology of developing striatal grafts, as seen in postmortem evaluation.^{24–30} Assessments of safety revealed that human fetal striatal grafts do not accelerate disease progression,^{25,31,32} and patients exhibiting no graft benefits appear to decline similarly to non-grafted patients.³³ The most convincing evidence of efficacy, to date, was demonstrated by Bachoud-Levi *et al.*,^{31,33} who reported on five patients who received fetal neural transplants. Benefits have been demonstrated both clinically and in terms of brain activity, lasting up to at least six years in some individuals.^{31,33,34}

Alternative cell sources

Fetal tissue as a source for cell replacement therapy is limited and is associated with both practical and ethical constraints. Trials, to date, have required multiple fetuses per patient, although the precise requirement is not determined as yet. This, in addition to the limited storage capacity of fetal tissue, poses logistical and quality-control issues for Good Manufacturing Practice (GMP) compatibility. Taken together, there is a pressing need to identify a renewable source of cells that are more readily amenable to the large-scale production of well-characterized GMP-compatible donor cells.

The requirement for any renewable donor cell source is to have the capability to generate the specific mature phenotype that is lost in the disease process. Specifically, for HD this means, as a minimum, generating cells that have a striatal MSN phenotype. It is also possible that in order to produce an optimally functioning graft, it will be important to include other striatal cell types. Thus, cells need to be responsive to inductive developmental cues, and the major challenge for the use of any donor cell is the specificity of the precise terminal phenotype. Potential renewable cell sources include expanded fetal neural precursors (FNPs), embryonic stem (ES) cells and induced pluripotent stem (iPS) cells.

Fetal neural precursors

FNPs are an attractive alternative because they are already lineage-restricted. Transplantation of human primary fetal

tissue, taken from the developing striatum, and short term expanded FNP (10 days) produced grafts with rich axonal outgrowth and cells expressing the neuronal marker neurofilament.³⁵ Long-term *in vitro* expansion of both mouse and human fetal neural progenitors prior to transplantation resulted in reduced survival following a long-term post-transplantation period.³⁶ It has been reported that there are changes in gene expression between early (4–8 weeks) and late (20 weeks) *in vitro* expansion times of human FNP, with changes in genes indicative of positional identity and up-regulation of genes associated with apoptosis after prolonged time in culture.^{36,37} Further, it has been shown that while the FNP phenotype of these cells following *in vitro* expansion may appear to be stable in terms of expression of certain markers, such as *Nestin* and *Sox2*, this expansion does result in reduced neuronal differentiation postgrafting, and has a negative impact on their ability to form viable grafts^{36,37} (unpublished observations). Thus, the longer striatal cells are expanded in culture prior to transplantation, the greater the need for directed differentiation towards an MSN phenotype.

ES cells

ES cells have a substantially greater proliferation potential than FNP, are more 'plastic' and are pluripotent.^{38–40} ES cells are derived from the inner cell mass of the developing blastocyst and have the potential to generate cells from any of the three primary germ layers of the embryo: ectoderm, endoderm and mesoderm. This makes them an attractive source of cells for therapeutic strategies in a range of diseases. However, one of the main caveats is obtaining the specific phenotype required, which in the case of HD, is MSNs. It is likely that the production of MSNs for HD transplantation will be a multistep process. Specifically, it will be necessary first to direct ES cells to a neuroblast suitable for transplantation. At this stage, full differentiation to a mature MSN phenotype *in vitro* prior to implantation would render the cell unlikely to survive the transplantation process. Thus, it will be necessary for the cell to continue differentiating to an MSN *in situ* in the graft.

There has been some degree of success in generating specific neuronal phenotypes from ES cells for replacement in other neurodegenerative diseases. Transplantation of ES cell-derived neural precursors has predominantly focused on obtaining dopaminergic neurons for Parkinson's disease (PD), and the general principles of directing differentiation of these cells applies for cell types throughout the developing brain. Dopaminergic neurons have been reported *in vivo* in graft-derived cells post-transplantation,^{41–43} with one case demonstrating long-term survival and function of these neurons.⁴⁴ However, although grafted cells have been shown to express dopamine, and partial improvements in amphetamine-induced rotations in animal models of PD have been observed, there is limited assessment of function post-transplantation to allow comparison of ES-derived dopamine cells to primary fetal tissue controls.

Directed differentiation of pluripotent cells to an MSN phenotype

Status of ES cell transplants in HD animal models

Few studies have been carried out using transplantation of ES cell-derived neural precursors into HD animal models. Of those that have been reported, only the QA lesion model has been employed. Table 1 outlines studies that have employed neural induction protocols for directing human ES cell-derived precursors for HD transplantation.

Mouse ES cell-derived precursors subjected to retinoic acid (RA)-induced suspension culture and then transplanted into the rat QA lesion model generated GABA- and AChE-immunopositive neurons following six weeks survival.⁴⁵ A study looking at the proportion of neural precursors, dividing cells and neurons, prior to transplantation, revealed that the greater the proportion of neural precursors (as determined by nestin immunoreactivity) and dividing cells (using bromodeoxyuridine), the greater the potential to generate tumors.⁴⁶ When the proportion of dividing cells was lower and that of mature neurons was greater, grafts generated neurons, astrocytes and oligodendrocytes, and there were no teratomas.

Human ES cell-derived neural precursors induced in defined neural induction medium, generated doublecortin (DCX)-immunopositive neuroblasts, neurons and astrocytes, but no DARPP-32-immunopositive neurons and no tumors at six weeks post-transplantation in a rat QA lesion model.⁴⁷ A similar study looked at neural induction of human ES cell-derived precursors with addition of Sonic Hedgehog (SHH) and Dickkopf-1 (DKK-1), and after six weeks survival, no DARPP-32 was found but there were teratomas.⁴⁸ Increased neural induction time with the addition of brain-derived neurotrophic factor (BDNF), dibutyryl cyclic adenosine monophosphate (dbcAMP) and valproic acid prior to transplantation resulted in generation of some DARPP-32-immunopositive neurons and no teratomas.⁴⁸ Longer-term survival up to 21 weeks post-transplantation of these latter neurally-induced precursors revealed regions of DARPP-32-immunopositive cells, but there was also the observation of large, overgrown grafts that compressed the host brain.⁴⁸

A recent study of human ES cell-derived precursors generated in neurobasal medium with basic fibroblast growth factor (bFGF) and the bone morphogenetic protein (BMP) inhibitor noggin, cultured for more than four weeks, expressed 80% nestin-positive cells at transplantation.⁴⁹ Relatively low numbers of cells were transplanted into the non-lesioned rat striatum and differentiation was analyzed at 1.5, 3 and 6 months. Nestin and DCX decreased over time post-transplantation (44–1% for nestin expression; 52–5% for DCX expression), while β -III-tubulin expression increased over time (20–86%), and although there was no DARPP-32 expression at three months, nearly a third of grafted cells were DARPP-32-positive at six months, demonstrating the need for long-term differentiation to achieve DARPP-32 expression.⁴⁹

A comparison of ES cell-derived neural precursors exposed to noggin compared with those grown in neurobasal medium alone, showed increased neuronal

Table 1 Transplantation studies of human ES cells into the striatum

Study	Cell line	Host	Protocols	Transplant	Results
Joannides <i>et al.</i> (2007) ⁴⁷	H9 HUES9	QA-lesioned striatum, rat, CsA	Defined neuralizing medium	100,000–250,000 cells transplanted Survival: 6 weeks	Grafted cells expressing DCX, NeuN, GFAP, but no DARPP-32
Song <i>et al.</i> (2007) ⁷⁷	Miz-hES1	QA-lesioned striatum, rat, CsA	Co-culture on PA6 stromal cells (generate high proportion of nestin-positive cells)	20,000 cells in 2 μ L transplanted Survival: 3 weeks	No functional assessment Grafted cells expressing nestin, Tuj1, DCX & GAD. No NeuN, Map2 or DARPP-32
Aubry <i>et al.</i> (2008) ⁴⁸	SA-01 H9	QA-lesioned striatum, immunocompetent rat	Rosettes in N2 & BDNF; addition of SHH/DKK-1; differentiation in BDNF/dbcAMP/valproic acid	50,000–200,000 cells transplanted Survival: 4–6 weeks	No tumors Improved apomorphine rotations at 1, 2 & 3 weeks compared with sham group Transplantation in early stages of neural induction resulted in teratomas & no DARPP-32 Transplantation of later stage cells resulted in no teratomas, and grafts contained clusters of DARPP-32 & calbindin neurons Large overgrown grafts that compressed the host brain. Regions of graft-derived DARPP-32 cells throughout the graft, often calbindin-negative, unlike short-term grafts
Nasonkin <i>et al.</i> (2009) ⁴⁹	BG01	Unlesioned striatum, nude rat	Neurobasal as above. Cells at the stage that gave best <i>in vivo</i> outcome at 4–6 weeks	50,000 cells transplanted Survival: 13–21 weeks	Very few TH neurons, no AChE & few GFAP cells No functional assessment
			Neurobasal with 7.5% KSR/bFGF/noggin for 3 days. Colonies picked from MEFs onto gelatin in neurobasal with bFGF/noggin for 4 weeks	15,000 cells transplanted Survival: 1.5, 3 & 6 months	High nestin expression prior to grafting (80%). Nestin and DCX expression decreased overtime <i>in vivo</i> , β -tubulin expression increased overtime. No GAD at 6 months; no DARPP-32 at 3 months but 30% DARPP-32 at 6 months; some calretinin & parvalbumin at 6 months No teratomas or graft overgrowth No functional assessment
Vazey <i>et al.</i> (2010) ⁵⁰	ENVY (GFP-expressing)	QA-lesioned striatum, rat, CsA	Neural precursors derived spontaneously or with noggin. Rosettes mechanically harvested & cultured in suspension in neural medium with growth factors for 3 weeks	75,000 cells transplanted Survival: 4 & 8 weeks	Grafted cells expressing Map2 and NeuN at 4 & 8 weeks, with more in noggin-primed grafts compared with spontaneously-derived grafts. No DARPP-32 or GAD67 Overgrowth in 1 graft from spontaneously derived group at 8 weeks No functional assessment

ES, embryonic stem; QA, quinolinic acid; DCX, doublecortin; DARPP-32, dopamine and cyclic adenosine 3',5'-monophosphate-regulated phosphoprotein, 32 kDa; BDNF, brain-derived neurotrophic factor; SHH, Sonic Hedgehog; DKK-1, Dickkopf-1; AChE, acetylcholinesterase; dbcAMP, dibutyryl cyclic adenosine monophosphate; GFAP, glial fibrillary acidic protein; TH, tyrosine hydroxylase; GAD, glutamate decarboxylase; NeuN, neuronal nuclei; CsA, cyclosporin A; KSR, knockout serum replacement; bFGF, basic fibroblast growth factor; MEFs, mouse embryonic feeders

differentiation post-transplantation.⁵⁰ Assessment of striatal phenotypes in the grafts revealed no expression of DARPP-32 or GAD-67.⁵⁰

Thus far, transplantation of ES cell-derived neural precursors has resulted in good survival in the QA-lesioned host brain, with good integration and neuronal generation, in some instances even into DARPP-32-immunopositive neurons. However, functional assessment has been very limited.

Developing protocols for the directed differentiation of ES cells for HD

Some signals indicative of brain regionalization and specification have been shown to be capable of directing ES cell differentiation towards a specific phenotype. For example, the addition of SHH and RA induced ES cells to differentiate into motoneurons,⁵¹ and thus provided proof-of-principle that knowledge of developmental biology could be applied to ES cell differentiation. Further, such neurons were shown to be functional and were specific to the medial motor column.⁵² Additionally, GABAergic and glutamatergic neurons capable of firing action potentials have been generated in defined serum-free culture conditions in the presence of SHH antagonist cyclopamine.⁵³ These examples demonstrate the potential to direct ES cells to differentiate into functional mature neuronal phenotypes.

Time and length of exposure to developmental factors are important when treating cultures to direct their neural differentiation. Early exposure to high RA concentrations (5×10^{-7} mol/L) has been shown to promote neural differentiation of ES cells,⁵⁴ but also results in complete loss of the forebrain markers *Foxg1* and *Otx2* and induction of caudal markers.^{55,56} Later addition results in suppression, rather than loss, of forebrain markers, but still with induction of caudal markers.⁵⁶ This supports the theory of specific time windows for addition of factors in influencing the fate of neural precursor cells.

When added to cultures of mouse ES cells, RA induces concentration-dependent effects on neural differentiation. *Ngn2* expression is enhanced by high RA concentrations, while at lower concentrations, mesodermal (*Brachyury*) and endodermal (*Pdx1*) markers are detected. It has also been demonstrated that higher RA concentrations induce a dorsal phenotype, while lower RA concentrations induce a more ventral phenotype, as indicated by expression of ventral genes such as *Nkx2.2*.⁵⁷ This induction of ventral neural progenitors with low RA concentrations occurred as a result of increased expression of the N-terminus of SHH protein, and the effect was abolished upon addition of cyclopamine.⁵⁷

The mouse ES cell culture system offers a robust model that is easy to manipulate, enabling integration of reporter systems for analysis of genes of interest in response to signaling cues. Although there are differences in the maintenance of mouse ES cells compared with human ES cells, their fundamental properties are transferable, including the capacity to respond to developmental cues. Some of the shared characteristics of mouse and human ES cells include expression of the ES cell marker Oct-3/4, telomerase

activity and the capacity to form teratomas containing derivatives of the three germ layers.⁴⁰

Various methods have been utilized for neural lineage derivation from human ES cells, including co-culture on stromal cells, which induces formation of neural rosettes, with subsequent culture of cells attached to substrate;^{58,48} stromal cell co-culture followed by suspension culture and then further differentiation on substrate;⁵⁹ and direct transfer of ES cells to defined neuralizing medium under substrate and feeder-free conditions.^{60,61} Stromal-feeder induction of neural precursors from human ES cells yields a decrease in expression of ES cell markers, such as Oct-3/4, with an increase in both Pax6 and MAP2 expression over time.⁵⁸ Neural rosettes following sequential application of SHH, FGF8, BDNF, ascorbic acid, glial cell line-derived neurotrophic factor (GDNF), dbcAMP and transforming growth factor- β 3 resulted in 30–50% β -III-tubulin-positive neurons, with 64–79% of these expressing tyrosine hydroxylase (TH) and 1–2% expressing GABA.⁵⁸ Application of a similar protocol of sequential addition of factors, but with the notable inclusion of DKK-1 and exclusion of FGF8 and GDNF resulted in 22% MAP2-positive neurons, of which 36% expressed GABA, 53% expressed DARPP-32, 10% expressed calbindin and less than 2% expressed TH.⁴⁸

Culture of human ES cells in suspension with defined human neuralizing medium, which included human serum albumin, transferrin and insulin, as well as amino acids and lipids, resulted in expression of immature neural markers including Sox1 and Pax6.^{47,61} Following 14-day terminal neuronal differentiation of these cells at day 25, 95% of the population expressed β -III-tubulin, and at day 70, 60% expressed β -III-tubulin.⁴⁷ Further analysis of the day 70 derived neurons revealed that 55% expressed GABA and 34% expressed glutamate.⁴⁷

A novel method for neural induction of human ES cells uses two inhibitors of Smad signaling (activated in the BMP signaling pathway), noggin and SB431542, which have previously been shown to enhance neural induction,^{43,62,63} in order to avoid the use of stromal feeder layers.⁶⁴ Addition of both inhibitors resulted in an increase in Pax6 expression to 80% of the total population by day 7 in culture. There was also an increase in the neural markers *Otx2*, *Foxg1* and Sox1, with a decrease in Oct-3/4 expression and by day 19, there was expression of the neuronal marker TUJ1 and the dopaminergic neuronal marker, TH.⁶⁴ The generation of such protocols helps to lead the way for more specific lineage induction of ES cells, while concomitantly avoiding potential contamination from feeder cells and associated medium components. However, further directed differentiation is needed for generation of striatal MSNs for HD.

One concern regarding neural induction protocols with ES cells is that differentiation is not exclusively neural and the resulting population is often heterogeneous containing multiple cell types. The continued presence of undifferentiated ES cells in the culture system will be a major concern for the therapeutic application of such cells and is the subject of ongoing investigation that lies beyond the scope of this review.

A key requirement in producing donor cells for therapeutic application is the reliable and reproducible production of a defined neuronal phenotype that not only has the capacity to survive, but also to function following transplantation into the brain. Although protocols have been developed which yield some DARPP-32-positive neurons, these are not yet proven to be fully functional, and quantity and reliability is not currently sufficient to progress to clinical trial. Thus, there is a pressing need for the development of further protocols to achieve full and robust MSN differentiation. Furthermore, since primary fetal tissue has provided the proof-of-principle for cell replacement studies and is the current 'standard', it is important when assessing alternative cell sources, to refer to primary tissue controls in order to draw comparisons.

iPS cells for HD

The emergence of iPS cells might prove useful for both research and clinical applications, since they bypass the use of embryos and would enable generation of patient-specific cell lines, therefore avoiding administration of immunosuppression. iPS cells have been shown to be capable of generating cells from the three germ layers and, with respect to neurally induced cells, expression of Pax6, Map2, β -III-tubulin and glial fibrillary acidic protein.⁶⁵ Differentiation of iPS cells towards a neural lineage using the dual Smad inhibition method generated dopaminergic neurons and motoneurons.⁶⁴ Indeed, PD patient-derived iPS cells have the capacity to differentiate into dopaminergic neurons and, following transplantation, developed limited projections to host striatum and brought about a degree of functional recovery.⁶⁶ HD patient-derived iPS cells have also been reported to generate neural precursors. Differentiation of these cells resulted in generation of striatal neurons showing expression of DARPP-32 as well as β -III-tubulin, GABA and calbindin.⁶⁷ More recently, direct conversion of fibroblasts into neurons, bypassing the pluripotent stem cell stage, has been reported.⁶⁸ Specific neuronal phenotypes have been achieved with this method, with different combinations of factors resulting in generation of dopaminergic neurons.^{69,70} This relatively new area offers a method of generating neurons without the pluripotent stem cell stage, potentially reducing or eliminating altogether the risk of teratomas. Whether this source can be of use for generation of cells for repair in HD is still to be determined, and integration into the host brain and appropriate functional recovery will need to be assessed following transplantation.

Need for markers of MSNs

Expression of DARPP-32 in ES cell-derived neurons following directed differentiation is encouraging. However, although being a reliable marker of mature MSNs *in vivo*, DARPP-32 is not exclusive to MSNs and, if used in isolation, could be misleading. This is especially the case when it is used to label cells derived from pluripotent stem cell sources, as opposed to cells derived from primary striatal

tissue where the cells have been exposed to developmental cues within the right brain region. Furthermore, DARPP-32 is not expressed in MSN precursors and is unreliable in culture.

Further markers of MSNs are continuously being sought, including those expressed in MSN precursors. A microarray analysis of gene expression in the developing mouse striatum identified genes that were altered during striatal neurogenesis (unpublished observations). One of the most significantly upregulated genes in this screen was *FoxP1*, which has previously been reported to be expressed in the developing striatum from E12.5 with continued presence in this region through to adulthood.⁷¹ In the striatum, *FoxP1* expression has been shown to be specific to the projection neurons but not interneurons of the adult striatum, with all DARPP-32-positive cells expressing *FoxP1*^{71,72} (unpublished observations).

FoxP1 has been shown to co-label with the transcription factor COUP TF1-interacting protein 2 (*Ctip2*).⁷³ Within the striatum, *Ctip2* has been shown to be expressed exclusively in MSNs, and all DARPP-32-positive cells co-expressed *Ctip2*.⁷³ However, expression of neither *FoxP1* nor *Ctip2* is restricted to the striatum and have been shown to be expressed in other brain regions including the cortex.⁷¹ Elsewhere, *FoxP1* is also known to be expressed in the lungs and heart.^{74,75} Additionally, in neurons derived from human ES cells, *CTIP2* has been shown to co-label with the dorsal telencephalic markers *TBR1* and vesicular glutamate transporter 1.⁷⁶

It would be valuable to define a profile of markers present in MSNs over the course of development, both in precursors and in terminally differentiated MSNs. This would enable identification of cells at different stages of differentiation, which would greatly facilitate the generation of protocols to produce MSNs. With increasing interest in the use of renewable cell sources for derivation of MSNs, a signature panel of markers becomes more important for progression of this field.

Final comments

There are still unanswered questions regarding differentiation of cells for repair in HD. Indeed, all the necessary requirements to produce the optimal graft for HD are not yet determined, even when the donor cells are primary fetal striatal neurons. Specifically, this includes the composition of the cell population to be grafted, be it a mixed neuronal population (projection neurons and interneurons) or a combination of neurons and glia, and the precise stage of development.

Currently, there is no protocol for robust, efficient and reproducible differentiation of MSNs either *in vitro* or *in vivo*. Central to the development of new protocols for directing the differentiation of cells to specific phenotypes is the understanding of signals that are important in their development in the brain. Characterization of MSN development will be a crucial step towards a better understanding of the factors that will be important in directing differentiation of cells towards this phenotype. A panel of

markers involved in MSN development is required in order to determine the correct lineage differentiation at various stages of differentiation. In addition, functional assessment of cells, using electrophysiological and calcium imaging methods, as well as assessment of graft-induced recovery, using behavioral analyses, should be included to develop a more stringent quality control so as to positively identify that the 'right' cell(s), and thus the optimum graft, has been generated. It should be reinforced that these indices should be compared with the current gold standard, which for HD transplantation is primary fetal striatal cells.

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