Emerging concepts in neural stem cell research: autologous repair and cell-based disease modelling

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The increasing availability of human pluripotent stem cells provides new prospects for neural-replacement strategies and disease-related basic research. With almost unlimited potential for self-renewal, the use of human embryonic stem cells (ESCs) bypasses the restricted supply and expandability of primary cells that has been a major bottleneck in previous neural transplantation approaches. Translation of developmental patterning and cell-type specification techniques to human ESC cultures enables in vitro generation of various neuronal and glial cell types. The derivation of stably proliferating neural stem cells from human ESCs further facilitates standardisation and circumvents the problem of batch-to-batch variations commonly encountered in "run-through" protocols, which promote terminal differentiation of pluripotent stem cells into somatic cell types without defined intermediate precursor stages. The advent of cell reprogramming offers an opportunity to translate these advances to induced pluripotent stem cells, thereby enabling the generation of neurons and glia from individual patients. Eventually, reprogramming could provide a supply of autologous neural cells for transplantation, and could lead to the establishment of cellular model systems of neurological diseases.

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Introduction

Neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, are characterised by progressive loss of neuronal subtypes over time. By contrast, loss of larger areas of CNS tissue is seen in acute lesions, such as in ischaemic or haemorrhagic stroke and spinal cord injury. Both cases require strategies to manage the neurological deficits caused by the tissue destruction. Current therapies, which are ineffective for several of these disorders, focus on symptomatic treatment with orally administered drugs to modulate concentrations of neurotransmitters, deep brain stimulation, or physiotherapy. In this Review, we discuss cell replacement and other cell-based therapeutic approaches as powerful alternatives, particularly for patients in whom symptomatic treatment is of limited benefit. To better understand the pathogenesis of neurodegenerative disorders and to find new drugs that prevent cell loss, cellular models that mimic the hallmarks of the particular disease in vitro are highly warranted. Here, we review the latest advances in neural cell generation, neurotransplantation, and cell-based disease modelling.

New sources for old tasks: the use of stem cells as a donor source for neural transplantation

Recent progress in stem cell research has opened new avenues to generate large numbers of different neural cell types in vitro and to use them for repair of the nervous system. In parallel, novel means for recruitment of endogenous neural stem cells (NSCs) into CNS lesions have emerged. However, these basic scientific advances still await successful translation into clinical practice.[1]

In the past, primary neural tissue obtained from post-mortem embryos has been used for transplantation, with some promising results in patients with Parkinson's disease and Huntington's disease.[2,3] These studies have provided evidence that functional restoration by neuronal replacement can work in the diseased human brain. However, many of the grafts did not survive and established only limited re-innervation; hence, in patients with Parkinson's disease, cell therapy has not yet been proven to be superior to clinically established approaches such as drug treatment or deep brain stimulation.[2,3] One major reason for the inconsistencies in clinical outcome might be the heterogeneity of the donor tissue, as cells from several donors need to be pooled for one transplant recipient.

To increase the availability of cells for transplantation and to standardise their quality, researchers have been looking for expandable sources of neural tissue for many years. Promising advances were made when embryonic neural cells or cells taken from neurogenic regions of the adult brain were shown to be expandable in vitro.[4–8] So far, expanded primary tissue has not been studied in clinical trials. A key restriction in the use of neural cells expanded by growth factors is that prolonged in vitro proliferation of neural precursors is associated with a decrease in their neurogenic potential and a concomitant increase in gliogenesis.[9] Furthermore, long-term expansion of neural cells in the presence of fibroblast and epidermal growth factors seems to bias the neuronal progeny of these cells towards an inhibitory GABAergic phenotype,[6,7,10] which might be due to loss of regional identity.[11] Alternatively, such a shift towards gliogenesis and the generation of GABAergic neurons might indicate a cell-autonomous temporal switch of NSC identity.[12] Evidence for such an endogenous timer mechanism was recently provided by Naka and co-workers,[13] who showed that the transcription factors Nr2f1 and Nr2f2 (of the nuclear receptor subfamily) are required for the temporal specification of neural stem or progenitor cells, including their acquisition of gliogenic competence.

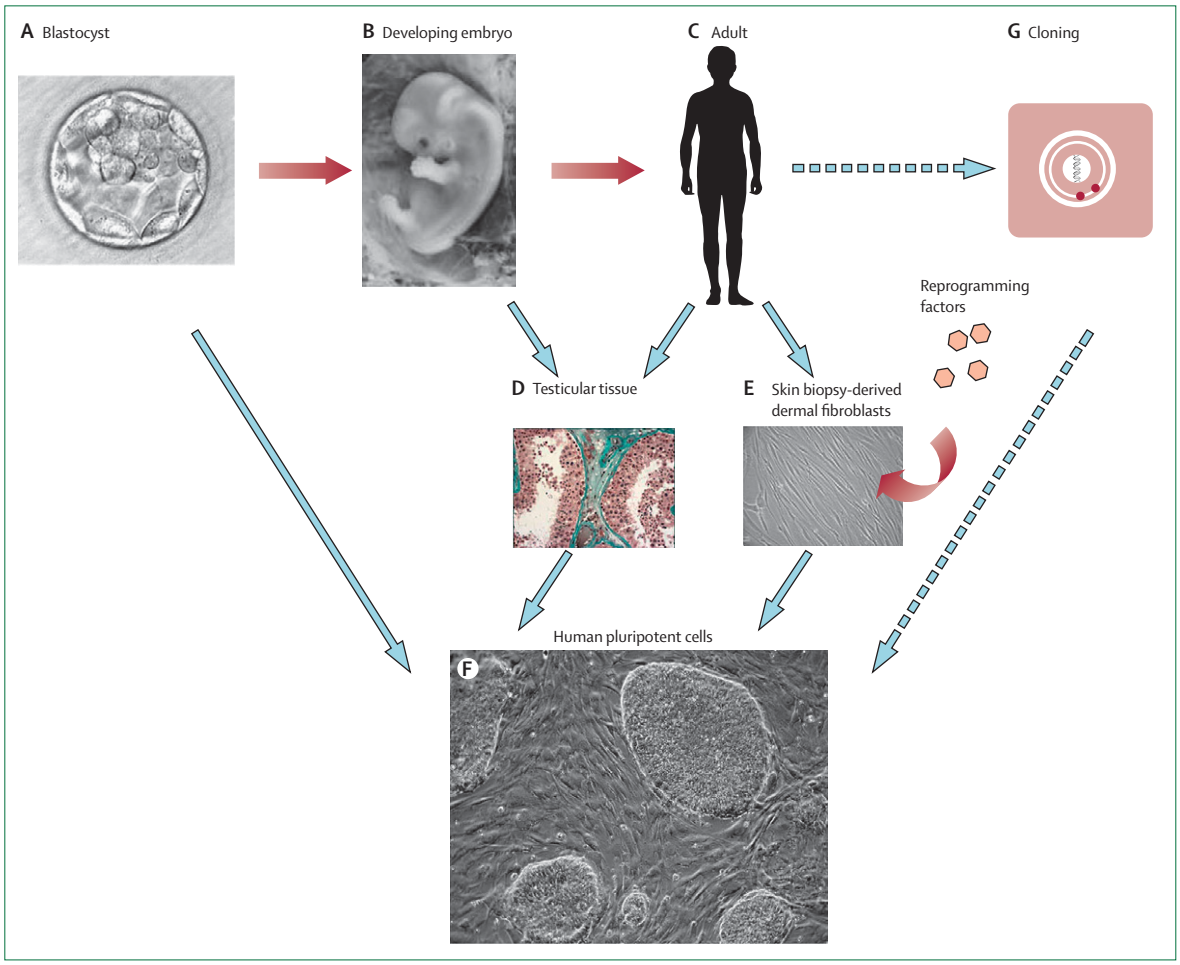

Figure 1: **Different sources of pluripotent cells**
Pluripotent human embryonic stem cells are derived from the inner cell mass of the early-stage human blastocyst (A, F).[14] In the developing embryo, primordial germ cells with characteristics of pluripotent cells were isolated (B, D, F).[15] Recently, a similar population of pluripotent cells was generated from adult testicular germ cells (C, D, F).[16-18] Exposure of adult somatic cells (eg, skin fibroblasts) to reprogramming factors enables the generation of induced pluripotent stem cells (C, E, F).[19-24] Human pluripotent stem cells shown in (F) were cultured on a feeder layer of murine embryonic fibroblasts. Other reprogramming techniques such as therapeutic cloning by somatic cell nuclear transfer or cell fusion have so far not been successfully transferred to the human system (G).

Embryonic stem cells: an unlimited donor source?

Because of the limitations associated with tissue-derived stem cells, pluripotent stem cells such as human embryonic stem cells (ESCs) are being increasingly discussed as a potential donor source for neural transplantation (figure 1). Human ESCs are derived from the blastocyst and can be proliferated almost indefinitely in an undifferentiated state.[14] Since 2001, when the first neural progenitors from human ESCs were described,[25,26] protocols have been improved so that different neural cell types can now be obtained from human ESCs in high purities.[27–32] These neural subtypes include dopaminergic neurons[27,29,30] (figure 2), motor neurons,[28,29] striatal interneurons,[32] and oligodendrocytes,[31,33] which are the main degenerating cells in Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis, respectively. Transplanted human ESC-derived dopaminergic neurons were shown to survive and contribute substantially to recovery of motor function in a rodent model of Parkinson's disease.[30,34] Transplanted motor neurons showed electrophysiological activity, as well as outgrowth of choline acetyltransferase-positive fibres, which caused clustering of acetylcholine receptors in co-cultured myotubes.[35] Grafted striatal progenitors developed into neurons expressing PPP1R1B (also known as DARPP32),[32] and human ESC-derived oligodendrocytes restored locomotion after spinal cord injury.[31]

In rodent models, ESC-derived neural cells have also been used for gene transfer into the CNS, with the aim of enabling local delivery of trophic factors or substitution of deficient enzymes. Overexpression of human aryl-sulfatase A in ESC-derived glial progenitors was sufficient to reduce sulfatide deposits in an animal model of metachromatic leucodystrophy.[36] In another study, ESCs deficient in adenosine kinase were used to generate

neural cells that released high levels of the inhibitory neuromodulator adenosine. On transplantation into a rat kindling model of epilepsy, these cells inhibited epileptogenesis and suppressed generalised seizures.[37]

Challenges associated with therapeutic use of ESCs

The successful use of ESCs in several rodent disease models might at first suggest that translation of human ESCs to the clinic is possible in the near future. However, several challenges need to be resolved. There is increasing evidence that ESCs are genetically and epigenetically unstable.[38–40] As a consequence, human ESC lines can vary substantially with regard to differentiation potential.[41] The availability of high-resolution genetic and epigenetic profiling methods might facilitate the selection of cell lines suitable for therapeutic and other biomedical uses. Another problem is that most of the established cell culture protocols are not adapted to Good Manufacturing Practice,[1] and include, for example, co-culture with murine fibroblasts or stromal cells to promote maintenance of pluripotency or dopaminergic differentiation, respectively.[27,30,32] Furthermore, most differentiation procedures represent "run-through" protocols, in which pluripotent cells are sequentially propagated in different media and growth factor conditions until they acquire the desired phenotype. Such approaches are prone to include undifferentiated human ESCs into the target population, which can result in formation of teratoma in the transplant recipients. A second risk factor for tumour formation is the protracted proliferation and differentiation times observed in human neural cells. This can lead to continuation of cell proliferation after transplantation, resulting in neural overgrowth.[30,32,42] Together, these concerns emphasise the need for more basic research into how to control proliferation and differentiation of ESCs and their neural derivatives before these cells can be considered for clinical use.

Despite the privileged immune status of the CNS, allogeneic grafts of stem cell-derived neurons and glia remain susceptible to rejection. One possible solution to this problem could be the establishment of ESC banks that contain ESC lines of different HLA haplotypes. On the basis of the assumption that the donor embryo HLA haplotype shows a random distribution, 150 ESC lines were calculated to provide a full match for HLA-A, HLA-B, and HLA-DR for less than 20% of the UK population, and a beneficial match for two haplotypes for only 37·9% of the population.[43]

Induced pluripotent stem cells: an autologous donor source?

An alternative strategy to avoid graft rejection and immunosuppression is the derivation of autologous pluripotent stem cells (figure 1). In 2006, Takahashi and Yamanaka[19] showed that skin fibroblasts from adult mice can be reprogrammed to a pluripotent state by retroviral

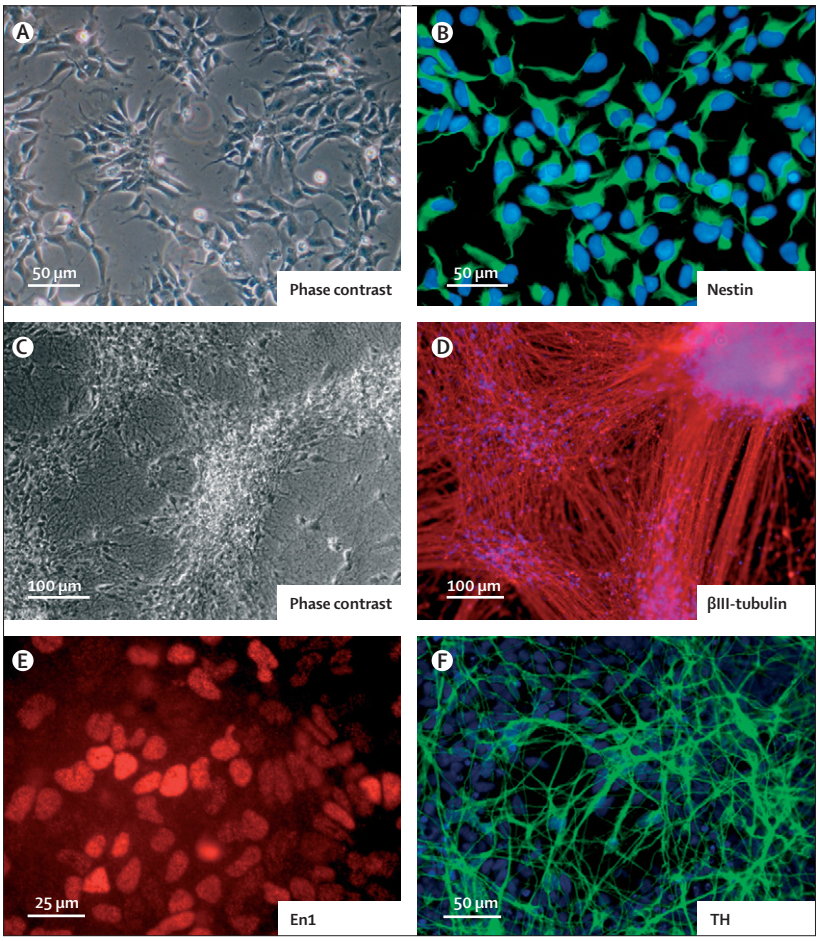

Figure 2: **Differentiation of human embryonic stem cell-derived neural stem cells into dopaminergic neurons**
A population of stably proliferating neural stem cells from human embryonic stem cells (A; phase contrast). These cells show homogeneous expression of the early neural marker nestin (B). On withdrawal of growth factor, neural stem cells give rise to a dominant fraction of cells with neuronal morphology (C; phase contrast), which express the neuron-specific marker βIII-tubulin (D; immunofluorescence). Exposure to the morphogens sonic hedgehog and fibroblast growth factor 8 enables the generation of ventral midbrain phenotypes that express the midbrain-specific transcription factor En1 (E). These cells can be differentiated into dopaminergic neurons that express TH (F). Adapted from Koch and co-workers.[29] En1=engrailed 1. TH=tyrosine hydroxylase.

expression of four transcription factors: Pou5f1 (also known as Oct4), Sox2, Klf4, and c-Myc. These induced pluripotent stem cells (iPSCs) give rise to all three germ layers, form teratomas, and contribute to chimeras and the germ line[20,21]—criteria typically used to confirm pluripotency of ESCs. Less than 2 years later, these findings were translated to the human system.[22–24] Meanwhile, the procedure to generate iPSCs has been continuously improved, for example by reducing the number of reprogramming factors and by the identification of small molecules that enhance reprogramming efficiency.[44,45] A major challenge is to generate iPSCs without the use of integrating viruses, which, owing to their oncogenic transgenes and insertional mutagenesis, carry the risk of tumorigenesis. Recently, several alternative techniques have been developed, including the use of non-integrating viruses,[46]

episomal expression systems,[47] excisable vectors,[48–50] and direct delivery of the reprogramming factors as bioactive proteins.[51,52]

The initial studies lend support to the idea that cells generated from iPSCs might be useful for transplantation. Wernig and co-workers[53] showed that mouse iPSC-derived dopaminergic neurons improved functional deficits in a rat model of Parkinson's disease. However, as most neurodegenerative diseases have a genetic component, generation of cells from the affected patient might, in some cases, require genetic correction before transplantation. Along this line, in a recent proof-of-concept study, Hanna and colleagues[54] used gene targeting to repair the genetic defect in iPSCs derived from mice with sickle cell anaemia. Haematopoietic progenitors generated from these gene-corrected iPSCs were indeed able to rescue the disease phenotype when transplanted back into the affected mice. Thus, this technique, celebrated by *Science* as "breakthrough of the year" in 2008,[55] might, in the long term, provide an avenue to derive customised and patient-specific cells for autologous transplantation.

The reprogramming technology could provide substantial advantages in the selection of immunocompatible allogeneic donors. If donors for the generation of iPSCs could be pre-selected from blood banks, ten highly selected donors might suffice to fully match 37·7% and beneficially match 67·4% of all recipients.[43] Similarly, 30 iPSC lines would deliver a full match for 82·2% of the Japanese population.[56]

Despite rapid progress in the field of reprogramming, several obstacles need to be overcome before iPSCs can be considered in a clinical context. First, standardised protocols have to be developed, yielding fully reprogrammed iPSCs without integration of foreign DNA. Second, genetic and epigenetic instability—already observed in cultured ESCs—will also be highly relevant for the use of iPSCs. For example, some studies have shown that more than 1200 genes are differentially expressed in human ESCs and human iPSCs,[22] and that iPSCs have an overall increase in DNA methylation.[57] Third, the optimum source of somatic cells used for reprogramming has not yet been identified. In addition to the risks associated with the use of oncogenic retroviruses, the choice of somatic cells used for reprogramming might be a crucial determinant for the probability of tumorigenicity.[58] Having been exposed to ultraviolet light irradiation and other damaging stimuli affecting genomic integrity, keratinocytes and skin fibroblasts might no longer be a suitable source. Finally, reprogramming affects only the nuclear genome, leaving mitochondria unaltered. The extent to which an aged and altered mitochondrial genome will affect the biology of iPSCs and their derivatives is unclear.

Germ cells: a novel source of neurons?

Another emerging source of autologous pluripotent stem cells could be germ cells (figure 1). Destined to give rise to gametes, these cells can, under certain circumstances, acquire a multipotent phenotype and form multiple germ layers (as is the case for patients with testicular teratomas). Following the discovery that primordial germ cells of mice can be transformed into an ESC-like state and give rise to all three germ layers,[15] protocols to derive multipotent germ cell-derived progenitors were sequentially translated to neonatal[59] and adult mice,[16,17] and eventually also to human beings.[18] Recent data show that adult unipotent germline stem cells derived from mice can spontaneously convert into a pluripotent phenotype.[60] Furthermore, there is evidence that stem cells derived from adult mouse testes can generate functional neurons and glia.[60,61] In human cells, this approach might provide an alternative route for the production of neural donor cells without complex reprogramming technologies.

Stem cell transplantation for Parkinson's disease

Stem cell therapies for neurodegenerative disorders are particularly suitable to test in Parkinson's disease because the main pathology is a selective loss of nigrostriatal dopaminergic neurons, providing a rationale for dopaminergic neuron transplantation. Moreover, data from clinical trials with intrastriatal transplantation of human primary embryonic mesencephalic tissue have shown that grafted dopaminergic neurons can re-innervate the denervated striatum and become functionally integrated, restore striatal dopamine release, and give rise to clear clinical benefit in some patients.[62] However, transplantation of primary neural tissue will not become routine treatment for Parkinson's disease owing to problems with availability and the large amount of variation in functional outcome. Stem cell technology might solve these problems.

Can stem cell-derived dopaminergic neurons function in animal models of Parkinson's disease?

To induce substantial clinical benefit, the grafted stem cell-derived dopaminergic neuroblasts must have the properties of substantia nigra neurons and, most likely, will need to be of human origin. Dopaminergic neuroblasts generated from several different stem cell sources have been tested in animal models of Parkinson's disease. These sources include mouse,[63–67] monkey,[68,69] and human ESCs,[30,70] rat[71–73] and human NSCs[74] derived from embryonic ventral mesencephalon, rat adult NSCs from the subventricular zone,[75] rat bone-marrow stem cells,[76] and mouse fibroblast-derived iPSCs[53] (figure 3). By overexpressing transcription factors that determine mesencephalic dopaminergic neuron specification or maturation during normal development, the yield of dopaminergic neurons with the correct phenotype from stem cells has been increased.[67,73,77,78] Enrichment of dopaminergic neurons (to >90% purity) has also been achieved by fluorescence-activated cell sorting of mouse ESC-derived

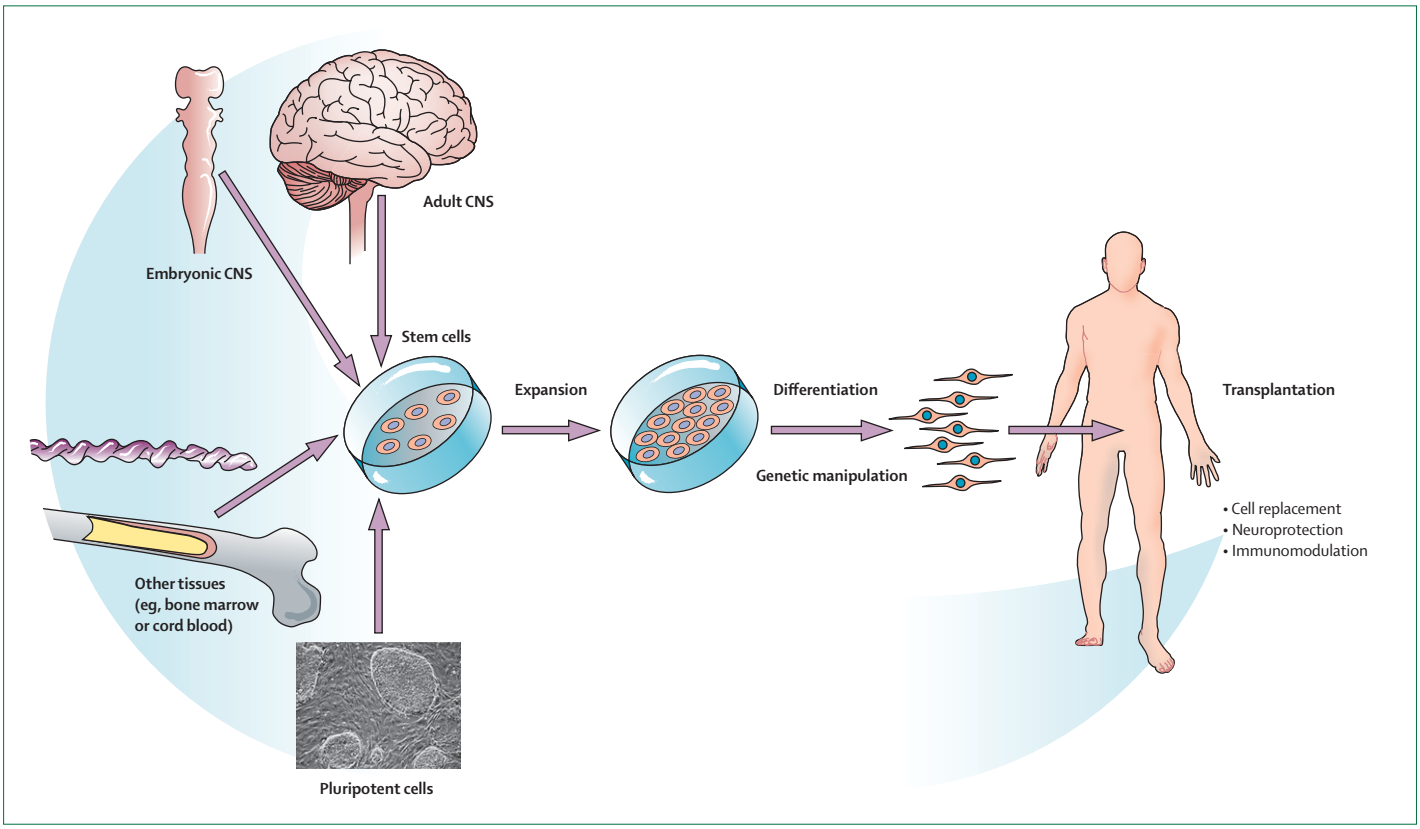

Figure 3: **Donor sources and approaches for neural transplantation**
Recent advances have provided different options for both donor sources and treatment regimens. Whereas traditional approaches mainly used embryonic donor tissue, the current experimental choices also include adult neural cells, non-neural somatic stem cells (eg, from bone marrow or cord blood), and pluripotent sources such as human embryonic stem cells and induced pluripotent stem cells. With regard to application, therapeutic benefits can occur through classic cell replacement, but also by transplant-mediated neuroprotective and immunomodulatory effects.

dopaminergic neurons.[79,80] Survival was reduced but the enriched dopaminergic neurons were functional after transplantation in a rat model of Parkinson's disease.

Are stem cell therapies ready for clinical use in Parkinson's disease?

Although stem cell-derived dopaminergic neurons can survive in animal models of Parkinson's disease and can exert some functional effects, dopaminergic neurons produced from stem cells have not been shown to be able to re-innervate the striatum, restore dopamine release in vivo, or, notably, improve deficits resembling the symptoms of patients with Parkinson's disease to the same extent as has been shown with primary embryonic tissue.[3] Experimental work to establish these important properties therefore needs to be done before stem cell-derived dopaminergic neuroblasts can be used for transplantation in patients.

A major concern when transplanting ESC-derived dopaminergic neuroblasts is the risk for tumour formation. Life expectancy is almost the same in patients with Parkinson's disease as it is for healthy individuals, making any risk unacceptable, even the minor risk of tumour formation associated with stem cell therapy. The risk for tumour formation in stem cell grafts and the consequences of the introduction of new genes must be carefully assessed in animals. Engineering of stem cells with regulatable suicide genes or use of cell sorting to eliminate cells that could give rise to tumours might be necessary. Therefore, transplantation of patient-specific dopaminergic neurons derived from iPSCs[53] or through therapeutic cloning[63] cannot yet be considered in a clinical setting. These patient-derived cells might also be more susceptible to disease pathology. In fact, Parkinson's disease pathology was recently reported to propagate from the host to the graft many years after intrastriatal implantation of primary mesencephalic tissue in patients.[81,82] However, cell therapy is still a viable therapeutic option because pathology develops slowly, most grafted dopaminergic neurons are unaffected after 10 years, and patients have long-term clinical improvements.

If a stem cell therapy should become clinically competitive with other treatment options in Parkinson's disease, the transplantation procedure will most likely need to be tailor-made with regard to dose and site of implantation of the dopaminergic cells on the basis of preoperative imaging. This approach enables the repair

of the dopaminergic system to be as complete as possible in each patient's brain. Patients with denervations restricted to the caudate nucleus and putamen will be likely to have major long-term benefits from dopaminergic grafts placed in these areas.[83] By contrast, long-lasting successful outcome in patients with Parkinson's disease with more widespread denervations (including, for example, the ventral striatum and cerebral cortex), will require grafts to be also placed in areas outside the caudate nucleus and putamen. The risk of troublesome off-medication dyskinesias after transplantation, which have been observed in a subgroup of patients with primary embryonic mesencephalic grafts,[84–86] needs to be minimised. This decrease in risk could be achieved by exclusion of serotonergic neurons from the graft[87] and by even distribution of the grafts over the putamen.

Parkinson's disease is not caused exclusively by dopaminergic deficiency, but is a complex, multisystem neurodegenerative disorder.[88,89] Therefore, therapeutic approaches that aim only to increase dopaminergic levels might not be sufficient to efficiently treat this disease. Owing to the heterogeneity of the population of patients, individualised treatment strategies that include oral medication, deep-brain stimulation, neural transplantation, or combinations thereof will most probably be required. Such approaches might also include stem cell-based delivery of compounds that can protect dopaminergic and non-dopaminergic neurons from degeneration in Parkinson's disease (eg, glial cell-derived neurotrophic factor).[90]

Stem cell transplantation for stroke

Compared with Parkinson's disease, in which the aim is to restore function by replacing only one specific cell type (ie, dopaminergic neurons), the challenge for stem cell therapy in stroke is much greater. This acute vascular disorder causes tissue loss, and many neuronal types as well as oligodendrocytes, astrocytes, and endothelial cells are destroyed. Stroke initiates activation of self-repair mechanisms comprising plastic changes at the synaptic level, reorganisation of existing and establishment of new neuronal circuits, and cell genesis, which can all contribute to recovery of neurological function. Support of these processes by supplying the brain with new cells to replace the damaged or dead ones or to act through other mechanisms could have important implications for recovery.

Can stem cells improve function in animal models of stroke?

Several types of stem cells have been successfully transplanted in rodent models of stroke, suggesting that they might also be suitable for use in patients.[33,91] Recent studies in stroke models have investigated the usefulness of human-derived stem cells, which would be required in a clinical setting. Human ESC-derived NSCs, grafted into stroke-damaged brains of nude rats, migrated towards the lesion and improved the function of the impaired forelimb.[92] After genetic modification, the grafted cells could be monitored for up to 2 months with bioluminescence and MRI.[93] Electrophysiological recordings showed that the grafted cells developed sodium currents and received synaptic input from host neurons,[93] similar to what was observed for mouse-derived ESCs grafted in stroke-damaged rat brains.[94] As an alternative to human ESCs, Kallur and co-workers[95] isolated NSCs from human embryonic striatum and cortex—these cells generated morphologically mature neurons after transplantation in stroke-damaged rat striatum.[96] The striatal and cortical NSCs maintained the region-specific phenotype,[95,96] even after their neurogenic potential had been enhanced by overexpression of the transcription factor Pax6.[97]

Systemically delivered human-derived NSCs and mesenchymal stem cells can reverse post-stroke functional impairments by mechanisms other than neuronal replacement (figure 3). The improvement induced by the NSCs in rats was most likely due to an anti-inflammatory action, which was abolished after splenectomy.[98] When the NSCs were genetically modified to overexpress vascular endothelial growth factor or an antiapoptotic signalling factor, these cells promoted angiogenesis and enhanced survival, respectively, further improving functional recovery in mice.[99,100] Intravenously delivered human-derived mesenchymal stem cells, isolated from adult bone marrow, ameliorated functional deficits after stroke in rats by angiogenesis and neovascularisation, and improved regional cerebral blood flow.[101] Recently, mesenchymal stem cells derived from human ESCs were shown to migrate to the infarct region and express neuronal and endothelial cell markers when injected into the femoral veins of rats after stroke.[102] Infarction volume in the rats that received mesenchymal stem cells was smaller and behavioural recovery was better than in the control group.

Are stem cell therapies ready for clinical use in stroke?

Clinical trials with cell therapy have already been initiated in patients with stroke.[103] Using grafting to the infarcted area of an immortalised human teratocarcinoma cell line, phase I/II studies were done in patients with ischaemic or haemorrhagic infarcts affecting the basal ganglia and, in some cases, the cerebral cortex as well.[104–106] The slight improvement in some of the patients correlated with increased metabolic activity at the graft site. A randomised controlled phase I/II clinical trial has also been undertaken with autologous mesenchymal stem cells, which were intravenously injected in patients with ischaemic lesions in the middle cerebral artery territory.[107] No adverse effects or functional improvement were observed. Recently, the UK-based company ReNeuron received approval from regulatory authorities to start a clinical trial in patients with stroke to study transplantation of clonal, conditionally immortalised NSCs isolated from

For more on **ReNeuron** see http://www.reneuron.com

	Primary tissue	Embryonic stem cells	PGD-embryonic stem cells	Induced pluripotent stem cells
Advantages	Only moderate ethical concerns about derivation process	Unlimited expandability Broad patterning potential	Unlimited expandability Broad patterning potential Intrinsic expression of disease-associated genes	Derivation without ethical concerns Unlimited expandability Broad patterning potential Intrinsic expression of disease-associated genes Possibility to include sporadic forms of the disease
Disadvantages	Limited access Limited expandability Limited patterning potential Transgene overexpression mostly required Bias towards glial differentiation Limited to candidate gene approaches	Ethical concerns about derivation process Transgene overexpression required Genetic and epigenetic instability Limited to candidate gene approaches	Ethical concerns about derivation process Genetic and epigenetic instability Limited to candidate gene approaches	Genetic and epigenetic instability

PGD=preimplantation genetic diagnosis.

Table: **Advantages and disadvantages of the different donor sources for the establishment of cell-based models of neurodegenerative diseases**

human fetal cortex. Transplantation of these cells in a rat model of stroke induced improvements in sensorimotor function and gross motor asymmetry,[108] possibly through secreted factors promoting the growth of new blood vessels and improvement of cerebral blood flow. However, many questions remain to be solved in basic research and clinical settings before stem cell therapy can be advanced to full-scale clinical trials in stroke.[33] These questions include, for example, the types of cells that are suitable for transplantation; how to control their proliferation, survival, migration, and differentiation; procedures for cell delivery; and selection, monitoring, and assessment of patients.

Stem cells as cellular model systems for neurological disease and drug development

Although many neurodegenerative diseases can be modelled in mice, these systems typically do not reflect the entire range of the disease phenotype at either the anatomical or molecular level.[109] The absence of pathological phenotypes in many transgenic animals has compelled researchers to consider differences between mice and human beings.[110] Indeed, promising effects of novel therapies observed in rodents are frequently not reproduced in human clinical trials.[111,112] Many examples indicate the differential vulnerability of human and rodent cells to human disease-related transgenes and toxins. For example, processing of amyloid-β, a key factor implicated in the pathogenesis of Alzheimer's disease, differs between mice and human beings, as well as between various cell types.[113] There is evidence that already subtle genetic differences between human and non-human primates (about 1·5% of the genome) result in major alterations in protein processing.[109,114]

The increasing availability of human neural cells has boosted interest in the development of cell-based disease models that recapitulate defined pathogenic steps at the molecular level. In principle, there are two avenues to generate donor cells for such models. One possibility is to introduce disease-specific mutations into primary human neural cells, thereby mimicking disease-specific pathological pathways.[115] However, genetic manipulation of primary neural cells by classic transfection methods is challenging as these cells grow slowly and transfection efficiencies are low. As an alternative, viral vectors have been successfully applied to overexpress foreign genes.[115,116] Another limitation is the fact that primary cells expanded in growth factors for extended periods of time have diminished response to morphogens and are thus more difficult to recruit into region-specific cell types required for studying a particular disease (table).[109,115]

The availability of ESCs might enable the generation of more stable systems. In recent years, numerous protocols have been developed that enable the differentiation of ESCs into disease-associated neuronal subtypes. However, only a few reports on transgenic disease-specific human ESC lines are available. Schneider and co-workers[115] showed that overexpression of mutant α-synuclein elicits more toxicity in human ESC-derived dopaminergic neurons than in primary or human ESC-derived GABA-ergic neurons. Another series of experiments investigated the role of astrocytes in the pathogenesis of amyotrophic lateral sclerosis.[117,118] Specifically, human SOD1 (superoxide dismutase 1) mutant astrocytes induced neurotoxic damage when co-cultured with human ESC-derived motor neurons, but not interneurons. These data indicate the power of cell-based disease models for studying the differential vulnerability of diverse neural subtypes to pathogenic factors and the delineation of cell-autonomous versus non-cell-autonomous mechanisms of neurodegeneration.

A general factor to be considered is that disease-associated genes are overexpressed in most transgenic models. This overexpression yields high levels of mutant protein, which might result in inadequate protein–protein interactions and in activation of signalling pathways not typical of the individual disease. Another disadvantage is that single genetic alterations are studied in an otherwise unaltered molecular and cellular context. However, many neurodegenerative diseases are based on several and mostly unknown molecular and cellular alterations. Obviously, this multitude of alterations cannot be modelled via expression of individual disease genes in a candidate approach.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms "cell-based therapies", "embryonic OR neural OR pluripotent stem cells", "iPS cells" (induced pluripotent stem cells), "restorative therapies", "neural transplantation", "Parkinson", "stroke" from January, 1975, to June, 2009. Only papers published in English were reviewed.

An emerging new avenue to deal with these disadvantages is the derivation of disease-specific cells from the patients' own tissue (table). Until recently, such a strategy was limited by the fact that human neural tissue of diseased patients, if accessible at all, mostly represented non-expandable post-mortem specimens of advanced stages of the disease. Neural tissue from aborted embryos carrying chromosomal aberrations or inherited forms of neurodegenerative disorders has been suggested as an alternative potential donor source.[119] Human ESCs derived from embryos discarded in the process of pre-implantation genetic diagnosis (PGD; table) have emerged as a means to generate disease-specific pluripotent cells.[120] However, generation of such cell lines requires careful pre-screening and, owing to ethical reasons, is prohibited in many countries.

Considering these drawbacks, the fact that efforts to generate human cell-based disease models has increasingly focused on iPSCs is not surprising. The iPSC approach also has the advantage that cells can be generated retrospectively from patients who have a particular disorder, which, in principle, should enable correlation of the clinical history with the cellular phenotype. One of the main advantages of iPSC-based disease modelling is that this approach could also provide access to sporadic and genetically complex diseases that are not accessible to candidate gene-based transgenic models.[48] This is of particular importance as only about 10% of all patients with amyotrophic lateral sclerosis, 7% of patients with early-onset Alzheimer's disease, and less than 1% of all patients with Parkinson's disease are regarded as having familial variants, with only a subset of these patients carrying known mutations.[121,122]

Recently, the generation of patient-specific iPSCs has been successfully applied to several neurological disorders, including amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, Gaucher's disease, Down's syndrome, and spinal muscular atrophy.[48,123–125] Motor neurons differentiated from iPSCs from a patient with spinal muscular atrophy showed a significant decrease in size and number, decreased expression of *survival motor neuron 1* (*SMN1*), and delayed synapse formation compared with non-affected control cells. Neural cultures derived from these iPSCs showed increased expression of SMN1 after treatment with valproic acid or tobramycin, compounds known to increase concentrations of SMN1.[125]

Most neurodegenerative diseases appear late in adulthood, progress slowly, and can depend on environmental components. A key question is whether in vitro culture of iPSC-derived neurons and glia provides a sufficiently broad time window to detect disease-specific cellular changes. If not, transplantation into immunodeficient rodents and subsequent long-term follow-up of the human cells within the host brain might be required to study disease-specific cellular alterations.

Although iPSC-based disease models still await further validation, such model systems could not only facilitate basic research into molecular disease mechanisms in human beings, but could also provide useful tools for the direct assessment of the effect of various compounds on the pathogenic processes.

Conclusions

Although traditional cell replacement remains a central goal in applied stem cell research, recent progress in the derivation of patient-specific stem cells indicates that stem cells might be equally useful for disease-related research. Translation to human cell systems is no longer viewed solely as a prelude to clinical use—increasingly, it is regarded as an avenue to study the pathogenesis of complex neurodegenerative diseases and to identify novel therapeutic targets in highly controlled cell-based in vitro models. In parallel, the range of potential clinical stem cell uses is broadening to accommodate, in addition to classic cell replacement, neuroprotective and immunomodulatory strategies. Contributing to both our understanding of disease mechanisms and the development of new therapies, stem cell research is expected to continue to increase its importance in the field of neurology and other medical disciplines.

Contributors

All authors contributed to the literature search and the writing of the Review, as well as to the preparation of the table. PK and ZK prepared the figures. All authors have seen and approved the final version.

Conflicts of interest

OB is a scientific director and a shareholder in Life & Brain GmbH, a commercial research platform affiliated to the University of Bonn and the University Hospital Bonn. Life & Brain focuses on the development of novel strategies for the diagnosis and treatment of nervous system disorders using various approaches, including stem cell research. Life & Brain receives financial support from the state government and the University of Bonn. Profits flow back to the platform to cover and expand translational research activities. The major shareholders of Life & Brain are the University of Bonn and the University Hospital of Bonn. Under current regulations, Life & Brain's private shareholders do not receive any revenue from their shares. All other authors have no conflicts of interest.

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