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Genetically modified stem cells for the treatment of neurological diseases

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1. ABSTRACT

The central nervous system has a very poor regenerative potential and is difficult to access. This partly explains why neurological diseases often lack appropriate therapeutic options and represent the most significant burden for healthcare systems. Progress in understanding the molecular background of neurological diseases requires innovative approaches offering new hope for the patients. One of the most intriguing and promising options is the combination of stem cells with gene therapy. Unlike fibroblasts, stem cells exhibit a high tropism for disease-affected tissue and integrate into the nervous tissue. This makes them ideal candidates for the production and delivery of molecules of interest for treating the nervous system. This article reviews the methodology for obtaining pluripotent stem cells (iPSCs) as precursors for neuronal cells, glial cells and the current state of the art in applications of genetically modified stem cells in animal models of neurodegenerative diseases, stroke, axonal damage, tumors and epilepsy.

2. INTRODUCTION

It has been estimated that 25% of citizens suffer from some form of brain disorder which encompasses all mental health and neurological disorders, including neurodegenerative conditions (1). Although the mortality of stroke and neurodegenerative diseases is comparable to that of heart ischemia and malignant tumors, a high rate of life-lasting disability is often a consequence of neurological diseases. Therefore, there is an urgent need for innovative therapeutic options aiming to bring new hope for still incurable pathological conditions. Among some of the most promising concepts is the possibility offered by genetic engineering, by which a cell can be instructed to produce therapeutic molecules of interest. At the same time, the scientific breakthroughs in the field of isolation of undifferentiated pluripotent cells and the possibility to control *in vitro* differentiation initiated the stem cell era. Due to their capacity for self-renewal, it is possible to deliver large quantities of stem cells in their naïve state to the injury site. If their proliferative capacity is maintained,

cells may be prompted by the microenvironment to differentiate into the required cell type. If cells are obtained from later developmental stages, they may exhibit more mature intrinsic properties to transform them to cells of their target destination. On the other hand, one needs to consider possible risks of stem cell therapy: there are concerns that stem cells could proliferate excessively *in vivo* causing solid tumors (2). The fact that the more advanced the cells' developmental stage prior to transplantation, the more restricted is their ability to proliferate and form tumors *in vivo* suggests that tumorigenesis can be avoided by using pre-differentiated cells with restricted potential. Stem cells have several characteristics which make them suitable for the regeneration of the nervous system (3): 1) the ability to self-renew and differentiate into new neurons or glial cells, 2) easy propagation which allows genetic manipulations, 3) a high tropism for tissue affected by inflammation or by malignancies, which makes them ideal vehicles for the delivery of beneficial proteins, 4) the possibility to integrate into the host brain, and 5) the possibility to obtain autologous induced pluripotent stem cells (iPSCs), for example, from skin fibroblasts or from adipose tissue which can avoid both, rejection by the immune system and ethical problems. Together, the gene therapy approach and the developing stem cell field introduced the concept of regenerative medicine. Combining these two methodologies gives us a new powerful tool with high therapeutic potential.

3. GENETIC MODIFICATIONS AS A TOOL TO OBTAIN STEM CELLS

Until recently, pluripotent stem cells originated exclusively from the pre-implantation embryo inner cell mass, from which permanent embryonic stem (ES) cell lines are derived. The use of this material faces numerous hurdles, including ethical controversies and immunological obstacles. Therefore, alternative strategies were explored. A breakthrough came in 2006, when the generation of induced pluripotent stem cells (iPSCs) through the reprogramming of murine (4) and of human (5) adult somatic cells by retroviral integration of pluripotency-associated genes (Oct4, Sox2, Klf4, c-myc, Lin28 and Nanog) was reported. iPSCs, especially patient-specific iPSCs, share most features of human ES cells. They are molecularly and functionally quite similar (6), they circumvent immunological obstacles and they are less controversial than ES cells with respect to ethical considerations. Therefore, iPSCs offer unprecedented opportunities for biomedical research and clinical applications.

The major limitation of current reprogramming strategies with respect to medical applications is the chromosomal integration of viral vector genomes used to deliver the genes encoding reprogramming factors. This may cause insertional mutagenesis, unpredictable genetic dysfunction and residual expression of these genomes in the progeny of reprogrammed cells (5, 7, 8). Recently, it was shown that epigenetic reprogramming of somatic cells leads to appearance of mutations and aberrant reprogramming of DNA methylation (9, 10). Although it seems that iPSCs in culture select rapidly against mutated cells (11), extensive genetic screening should become a standard procedure to ensure cell safety before clinical use. A number of modified genetic methods have been developed and produced iPSCs with potentially reduced risks, including single viral cassettes, non-integrating adenovirus vectors, synthetic mRNAs, transient plasmid transfections, transposons, Cre-excisable vectors, and oriP/EBNA1-based episomal expression systems (12-17). However, these methods have very low reprogramming efficiencies and they still involve the use of genetic material and thus still are exposed to the risks associated with genetic modifications. To avoid genetic changes in reprogrammed cells, several groups identified small molecules that enhance re-programming and/or functionally replace some of the re-programming factors. Those factors include direct epigenetic modifiers, as well as signalling pathway modulators, such as MAPK inhibitors, GSK3beta inhibitors, and TGFbeta pathway inhibitors (18). So far, at least one transcription factor, Oct4, is still required to generate iPSCs (19). Another possible way to avoid introducing exogenous genetic material consists of the delivery of reprogramming proteins to target cells. These proteins can be conjugated to cell-penetrating peptides (CPPs) that represent suitable vectors for such purpose. The Antennapedia homeodomain, also called Penetratin, and the HIV Tat protein are CPPs formed of highly basic amino acid sequences that cross membranes. Other CPPs are artificially designed highly cationic and hydrophilic arginine-rich peptides (20). Two publications report successful reprogramming of primary mouse and human fibroblasts with proteins fused to CPPs (21),(22). However, these approaches require complicated cell culture conditions and suffer from very low efficiency. Nevertheless, proof-of-concept exists and we expect that these technical hurdles will be resolved soon.

4. NEURONAL DIFFERENTIATION OF STEM CELLS

One of the key challenges for translating stem cell therapies into the clinic is devising robust protocols for differentiating stem cells to lineage-committed cells. During embryonic neurogenesis, neural induction is regulated by the coordinated actions of bone morphogenetic proteins (BMP) and Wnt-and fibroblast growth factors/insulin-like growth factors (FGF/IGF)-signalling pathways. The neural plate is then patterned by extrinsic morphogens along the rostro-caudal and dorso-ventral axis into discrete domains. *In vitro*, neural induction and specification of mouse ES cells follow the same cues to give rise to well-defined neuronal populations. Protocols reported the generation of several mouse neuronal subtypes including spinal motor (23), midbrain dopaminergic (24), hypothalamic (25) and cortical neurons (26, 27). Consequently, the same protocols were applied to generate these different neuronal subtypes from human ES and iPSCs, as these cells differentiate to neuroepithelial cells and neurons via the same transcriptional networks (28). However, the transfer of mouse protocols to human cells often necessitated some adjustments. So far, human iPSCs were successfully differentiated into spinal motor neurons

(29, 30), dopaminergic neurons (31), glutamatergic neurons (32) and neural crest cells (24). However, pure neuronal population is difficult to obtain. For example, published protocols for motor neuron induction yielded heterogenous cell populations with variable proportions of neural precursors, glial cells and motor neurons, the latter representing at best 40% of all cells (29, 30). Importantly, there is still some variability between human ES or iPSC lines. They mostly consist of differences in epigenetic markers, expression profiles and differentiation profiles. In particular, the transcriptional signature in the undifferentiated state and their ability to differentiate into neural tissue may vary significantly (28, 33), probably reflecting the heterogeneity in the way they were generated. The underlying molecular mechanism of reprogramming still remains unclear. This inconsistency needs to be taken into account in future studies.

Recently, it was demonstrated that it may be possible to generate neurons from other adult cells, without the intermediate iPSC state. Vierbuchen *et al.* (34) showed that a specific combination of neural-lineage-specific transcription factors allowed the conversion of fibroblasts into neurons. Only three factors, Ascl1, Brn2 and Myt1l, were sufficient to rapidly and efficiently convert mouse fibroblasts into functional neurons expressing a variety of neuronal markers and capable of firing action potentials. Whereas the iPSC approach necessitates the complete de-differentiation of cells to an ES-cell-like state and re-differentiation to an adult cell type, a time-consuming detour, trans-differentiation is rapid and induced neuronal (iN) cells are unlikely to form tumors. One can imagine that reprogramming into dopamine neurons or motor neurons could be possible via the addition of supplementary specific transcription factors. For example, we showed that genetic engineering with vectors encoding a specific combination of motor neuron transcription factors allowed the reprogramming and efficient differentiation of neural precursors into motor neurons *in vitro* and *in vivo* (35). Future studies will show whether it is possible to generate iNs from human cells, to produce specific neuronal subtypes and whether these neurons are sufficiently mature for transplantation. Another important step will be to generate iNs with transient and non-viral reprogramming methods similar to those used in iPSC generation.

5. GENETICALLY MODIFIED CELLS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

5.1. Neurodegenerative diseases

Neurodegenerative diseases (ND) are characterized by degeneration of neurons in various parts of the nervous system. Among the most prominent are Alzheimer disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. Although the exact pathophysiological mechanisms are still elusive, it is known that they all share distinctive features: gradual accumulation of misfolded proteins, acceleration of aggregate formation and impaired autophagy which leads to neuronal death (36).

Stem cell based therapy of neurodegenerative diseases has three main aims: 1) to replace dead and damaged neurons, 2) to decrease or prevent neuronal death (e.g. by secretion of neurotrophins which both reduce neuroinflammation and support neuronal survival), and 3) to enhance endogenous repairing process. Genetically modified stem cells furthermore boost these benefits by controlled overproduction of proteins of interest. Examples of application of genetically modified cells for the most common ND diseases are presented in Table 1.

5.2. Parkinson's disease

Among all ND diseases, Parkinson's disease is probably the most accurately understood. The fact that the symptoms are primarily caused by localized degeneration of dopaminergic neurons in the mesencephalic substantia nigra is suggesting that successful replacement of this cellular population may cure the patients. Since dopamine was required in the striatum, the first attempts in neuro-regenerative medicine started by transplantation of fetal tissue into this region. Implantation of fetal ventral mesencephalic cells into the caudate and putamen of PD patients provided a marked improvement in their clinical course mainly in younger patients (37, 38). On the other hand, some failures and graft-induced side effects have as well been reported (reviewed by (39)). Interestingly, there is an evidence that grafts do survive for up to two decades, although their beneficial effects are jeopardized by the progressive disease which spreads throughout the transplanted cells (40).

Therefore, apart from ethical and practical problems regarding the use of human fetal tissue, it has become obvious that there is a need for other approaches, e.g. involving transplantation of genetically modified stem cells. These transplants represent an alternative source of cells which produce dopamine or L-dihydroxyphenylalanine (L-DOPA), the dopamine precursor. The most straightforward approach was focused on introducing genetically engineered cell lines that overexpress tyrosine-hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. One of the first experiments with genetically modified cells was performed with fibroblasts (41) genetically modified to produce L-DOPA. Numerous consecutive studies with stem cells have been based on either rat or human TH - transduced mesenchymal stem cells (MSCs) (42), TH- and guanosine triphosphate cyclohydrolase 1 (GTPCH1) - transduced NSC (43, 44), and they all reported significant improvements in animal motor condition.

Another approach was based on genetically modified stem cells which produce neurotrophic factors that promote survival of dopaminergic cells. Again, inspired by pioneering experiments based on fibroblasts (45) some successful trials based on delivery of glial derived neurotrophic factor (GDNF) have been published: it has been showed that GDNF increases number of TH – positive fibers at the place of cell transplantation, which correlates to reduction of the symptoms (46). The third category of use of genetically modified stem cells in Parkinson's disease encompassed the transfection with

Table 1. Application of genetically modified stem cells in neurodegenerative diseases

Animal model	Cells	Application	Outcomes	Reference
Parkinson's disease				
6-OHDA rats	Human ESC overexpressing TH and GTPCH1	Stereotaxic injection into the striatum, $1x10^5$ cells	Reduced symptoms	(44)
6-OHDA rats	Human MSC overexpressing GDNF	Stereotaxic injection into the striatum, $1x10^5$ cells	Reduced symptoms, tissue regeneration	(46)
6-OHDA mice	Mouse NSC overexpressing WNT5a	Stereotaxic injection into the striatum, $1x10^5$ cells	Cellular and functional recovery	(48)
6-OHDA rats	NSC overexpressing NURR1	Stereotaxic injection into the striatum	Histological and behavioral improvements	(47)
6-OHDA rats	Rat BMSC overexpressing TH	Stereotaxic injection into the ventricle, 1x105 cells	Reduced symptoms, increased quantity of brain dopamine	(42)
6-OHDA rats	Human NSC overexpressing TH and GTPCH1	Stereotaxic injection into the striatum, $1x10^6$ cells	Functional recovery	(43)
Amyotrophic lateral sclerosis				
$SOD1^{G93A}$ rats	Human MSC overexpressing GDNF	Intramuscular injection, $1.2x10^5$cells, 2 administrations	Unchanged disease onset. Delayed disease progression up to 28 days	(52)
$SOD1^{G93A}$ rats	Human NSC overexpressing GDNF	Injections into L1-L4 lumbar spinal cord, 4 sites, unilateral $1,2\text{-}1,8x10^5$ cells/site	No effect on survival. No improved locomotor function	(54)
$SOD1^{G93A}$ rats	Human NSC overexpressing GDNF	Injections into L1-L4 lumbar spinal cord, 2 sites, bilateral $1.2x10^5$ cells/site	No effect on survival	(53)
$SOD1^{G93A}$ mice	Human UCB overexpressing VEGF165 and FGF-2	Intravenous delivery, $1x10^6$ cells	Not studied	(56)
$SOD1^{G93A}$ mice	Human NSC overexpressing human VEGF	Intrathecal delivery, $1x10^5$ cells	Delayed disease onset, prolonged survival by 12 days	(108)
$SOD1^{G93A}$mice	Human NSC overexpressing BDNF, IGF-1, VEGF, NT-3 or GDNF	Injections into cisterna magna or cerebral ventricles	Unchanged or decreased survival	(109)
Huntington's disease				
YAC 128 mice	Mouse MSC overexpressing either NGF or BDNF	Stereotaxic injection into the striatum, $3x10^5$ cells	Improved motor scores (BDNF)	(62)
Quinolinic acid lesion rats	Rat NSC overexpressing NGF	Stereotaxic injection into the striatum	Decreased cell loss and regenerative axon sprouting	(63)
Quinolinic acid lesion rats	Rat NSC overexpressing either NGF or BDNF	Stereotaxic injection into the striatum, $9x10^5$ cells	Decreased cell loss and lesion size (NGF)	(61)
Alzheimer's disease				
3xTg – AD mice	Mouse NSC and NSC BDNF -	Stereotaxic injection into the hippocampus, $3x10^5$ cells	Improved hippocampal synaptic density and congnitive parameters	(65)
APPswe-PS1DeltaE9 mouse	Mouse BMC EP2 -	Stereotaxic injection	Improved clearance of amyloid plaques	(66)
16 months old rats	Rat NSC overexpressing NGF	Stereotaxic injection into the nucleus basalis and septum	Decreased cell loss and improved behavior scores	(64)

genes driving an increased differentiation and improving functional integration of transplanted stem cells. As a consequence, a larger number of survived cells yielded increased levels of dopamine (47, 48).

5.3. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting cortical and spinal motor neurons. Progressive cell loss leads to gradual paralysis and death of the patient most commonly only a few years after onset of the first symptoms. Different populations of stem cells including MSCs, NSCs and umbilical cord blood cells (UCBs) were successfully transplanted into SOD1 (superoxide dismutase 1) rodent models of ALS (49). They exerted beneficial effects through differentiation into astrocytes and neurons, reduction of astrogliosis and release of trophic factors such as GDNF, insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF). Stem cell transplantation was rapidly translated into human trials but, unlike their initial success in experimental models, a lot of discrepancies were highlighted regarding their outcome in the clinic. This discrepancy illustrates the need to analyze in depth both experimental protocols and in particular, the pharmacokinetic parameters for drug delivery.

First attempts to transplant MSCs in ALS patients at the level of the thoracic spinal cord was described by Mazzini *et al.* and recently gathered in a report of Phase I clinical trials (50, 51). It thus appears that transplantation of autologous MSCs is a safe procedure with no serious adverse effects. Later it was realized that stem cells may serve as "Trojan horses" to deliver neuroprotective factors. Using this approach it was recently shown that muscular delivery of hMSC-GDNF prevents motor neuron loss, delays disease progression and increases overall lifespan in the SOD1 animal model of ALS (52). Human NSCs engineered to deliver GDNF and intrathecal transplantation of human NSCs overexpressing VEGF also increased survival of ALS animals for several weeks (53, 54). Our group recently demonstrated that *in vitro* MSCs and NSCs possess the ability to express a large number of growth factors required for neuronal survival (BDNF, GDNF, IGF-1, VEGF), once exposed to an ALS environment (55). These growth factors may act either in an autocrine manner by modulating the migration, survival and differentiation of

stem cells themselves or through a paracrine mechanism acting on damaged motor neurons in order to support cell survival and tissue regeneration. Illustrating this autocrine effect, Rizvanov *et al.* recently showed that unmodified human UCB cells differentiated into endothelial and microglial lineages after transplantation into SOD1 mice while the same cells genetically engineered to overexpress VEGF and FGF-2 exhibited preferentially an astrocytic differentiation (56).

5.4. Huntington's disease

Huntington's disease (HD) is a dominant neurodegenerative disorder, characterized by a polyglutamine expansion that leads to the production of mutant huntingtin protein. This results in the loss of medium spiny neurons (MSNs) within the striatum, progressive motor deficits and dementia. Similar to Parkinson's disease, cell based therapy for Huntington's disease started with transplantations of fetal tissue or stem cells in both, animal models (57, 58) and the patients (59, 60). The majority of these experiments reported significant improvements in motor and cognitive functions.

Genetically modified stem cells have been used in experiments for delivery of nerve growth factor (NGF) or BDNF via immortalized NSCs in the rat (61) and via MSCs in the mouse (62). Although both experiments reported significant motor improvements, the positive effects were assigned to the treatment with the growth factors, not to the integration of stem cells into the host brain. Some of the discrepancies could have been explained by the fact that in rats a quinolinic lesion model was used, while in mice the YAC genetic model for HD was applied. Nevertheless, both experiments reported reduced striatal degeneration and cell loss as well as a reduced activation of astrocytes and microglia. The positive effects of NGF in the quinolinic acid rat model have been reported by another group describing a significantly reduced volume of the lesion and regenerative sprouting of axons (63).

5.5. Alzheimer's disease

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases characterized by disabling dementia, the appearance of senile plaques and neurofibrillary tangles in affected brain regions. From a therapeutic point of view, AD represents a huge challenge, as neuronal degeneration is widespread, beginning in the hippocampus, cortex, amygdala and progressing to many other regions of the brain.

In one of the pioneering works, 16 month old rats received transplants of NGF-secreting immortalized neural progenitor cells, bilaterally in the nucleus basalis (Meynert) and in the septum. During the subsequent 9 months the animals with NGF-secreting grafts maintained a performance level not different from the 12-month-old control rats. In the same time the aged control animals developed the expected impairment in spatial learning in the water maze task (64). In the triple transgenic Alzheimer (3xTg-AD) mice NSC transplantation significantly rescued the spatial learning and memory deficits without altering Aß or tau pathology. The mechanism involved enhancement of hippocampal synaptic density mediated by BDNF. NSCs with a deleted gene for BDNF completely failed to improve cognition or restore hippocampal synaptic density (65). Recently, it was reported that transplantation of bone marrow stem cells devoid of microglial prostaglandin E(2) receptor subtype 2 into aged AD mice exhibited an improved clearance of amyloid pathology, suggesting alternative options for the application of genetically modified stem cells (66).

6. GENETICALLY MODIFIED CELLS FOR THE TREATMENT OF STROKE

Stroke is the leading cause of disability and the third leading cause of death in the western world following heart disease and cancer (1). Thus substantial advances in the prevention and treatment of stroke are of paramount importance. The possible therapeutic benefit of stem cells in stroke patients is substantiated by the post-stroke activation of endogenous NSCs in mice. These cells exit the rostral migratory pathway and are redirected toward the ischemic lesion (67). Moreover, the expected beneficial effects were reached even relatively late after the onset of the stroke. Cells were administrated not only within 3 days post-stroke (in the majority of pre-clinical studies), but sub-acute (1 week post-stroke) and chronic (>3 weeks post-stroke) delivery was demonstrated to be beneficial as well. The positive effects appeared not to be achieved by long term integration of stem cells as they could not be detected any longer in rat 6 months after grafting (68).

In order to optimize and enhance the therapeutic effects of stem cells in stroke models, they were genetically modified to produce larger amounts of factors expected to contribute to different aspects of recovery after stroke. This approach was followed in various ways, for example, applying bone marrow derived MSCs, which already secrete endogenous beneficial molecules. NSCs were used as well with the hope of combining their neuronal phenotype with additional secreted molecules probably exerting neurotrophic and/or neuroprotective effects on the surrounding brain tissues. Among the various secreted neurotrophic and/or neuroprotective factors NGF and/or noggin (69), BDNF (70), GDNF (71), neurotrophin 3 (72) and erythropoietin (73) were used for genetic modification. Another aim of these studies was to enhance tissue recovery, through enhanced angiogenesis applying VEGF and/or angiopoietin-1 (74), PlGF (75), and Hypoxia-inducible factor 1alpha (76). Invariably the published studies reported positive effects, which were more extensive than applying the corresponding non-modified stem cells alone. The studies report that ischemic lesions were smaller, functional recovery increased, survival and differentiation of stem cells improved and angiogenesis increased. In the surrounding tissue there was less apoptosis and the invasion of microglia was reduced.

Although it is doubtful if neurogenesis contributed to the reduced loss of neurons, genetic modification was used to promote neuronal differentiation of stem cells. One of the aims was to drive MSCs toward a neural phenotype. For this purpose the cells were modified

to overexpress neurogenin 1 and Notch (77, 78). Neuronal differentiation was enhanced and consequences of the ischemic lesions were reduced in these studies. Moreover, the late application of the Notch-modified-MSCs in chronic stroke (i.e. 1 month or even 42 days after medial cerebral artery occlusion) resulted in better recovery of the treated animals (79). Furthermore, genetic modification was necessary to immortalize NSCs obtained from human fetal brain. The immortalization enabled researchers to produce indefinite numbers of these cells and to characterize them in detail, which was a prerequisite for the currently ongoing clinical trials. Oncogenes used included v-myc (80) and c-myc, but also a conditional immortalizing gene, c-mycER^{TAM}(81). The use of this vector results in cell proliferation only in the presence of a synthetic drug, 4-hydroxy-tamoxifen (4-OHT), while in its absence the cells undergo growth arrest and differentiate into neurons and astrocytes. The beneficial effects of these modified cells were further enhanced by additional overexpression of BDNF (82) or VEGF (83). In other studies cell survival was enhanced by overexpressing Akt1, a serine/threonine kinase, promoting cell proliferation and exerting anti-apoptotic functions (83).

7. GENETICALLY MODIFIED STEM CELLS FOR TREATMENT OF AXONAL DAMAGE

Spontaneous regeneration of function and structure rarely occurs following nervous tissue injury. The most relevant factors contributing to this lack of recovery include tissue damage, glial scarring and myelin-dependent inhibition of axonal regeneration (84). Neurotrophic proteins or antibodies against inhibitory molecules have been applied to overcome these limitations. However, to achieve long-term and site-specific delivery of proteins to the injured brain and spinal cord, *ex vivo* gene therapy has been suggested as the method of choice (85). This approach involves removal of Schwann cells, fibroblasts, glia or stem cells from the host followed by genetic manipulation of these cells *in vitro*. Cells successfully incorporating the transgene are selected, expanded in culture and then grafted into or close to the lesion site without taking a risk of immunological rejection. This treatment provides high levels of localized growth factor to the site of injury to induce, for example, robust axonal growth after spinal cord injury (86).

Stem cells are attractive carriers for genes into the lesioned CNS to promote axon regeneration, for example, to enhance the level of chaperones or anti-apoptotic molecules at the injury site (87). In response to lesions, stem cells start to divide, migrate to the site of injury and differentiate into glial elements (88). A study which used mouse embryonic stem cells transfected with the cell adhesion molecule L1 reported enhanced neuronal survival and neurite outgrowth (89).

In addition to extracellular matrix or cell adhesion molecules, cytoplasmic proteins may be suitable targets for overexpression in neurons as well. For example, regeneration requires extensive microtubule assembly/disassembly dynamics. The total levels of severing proteins are lower in adult axons compared to growing axons, there are far fewer short microtubules and less robust microtubule transport. These findings imply that injured axons in the spinal cord cannot assemble their microtubules as readily as in the embryo (90). Restoring the levels of microtubule severing proteins to their juvenile levels through transplantation of genetically modified cells may be a fruitful avenue for augmenting regeneration of injured adult axons.

Therapeutic approaches to improve CNS regeneration will likely benefit by adopting some of the favourable properties exhibited in peripheral nerve lesion models. In contrast to the CNS, peripheral nerve injuries result in spontaneous regeneration, mainly due to the intrinsically supportive properties of Schwann cells (91). They actively promote axon growth by phagocytosis of nerve debris, production of neurotrophic factors and secretion of extracellular matrix molecules that support axonal regrowth (e.g. laminin). However, even *in situ*ations of satisfactory physical contact between lesioned peripheral nerve stumps, axonal sprouting and aberrant axon growth hinder regeneration and functionally correct pathfinding (92). It is possible that the protracted loss of axonal contact renders Schwann cells unreceptive for directed axonal elongation. Therefore, the distal denervated nerve environment could be supported by replacing host cells with stem cells.

Schwann cells over-expressing FGF-2 have been extensively investigated as tools to improve peripheral nerve regeneration, in particular, in combination with exercise which reinforced the beneficial effects of transplantation and FGF-2 gene therapy in peripheral nerve reconstruction approaches (93). Other studies utilize stem cells genetically modified to overexpress potent motor neuron growth factors, for example, GDNF. They are grafted into denervated nerves followed by cross-suture of regenerating nerves (94). These animals revealed improved regeneration of peroneal axons into the tibial nerve as revealed by axon counting and by the emergence of compound motor action potential in the foot muscles. Some of the most recent successes achieved include use of oligodendrocyte precursor cells transfected with ciliary neurotrophic factor (animals with injured spinal cord exhibited significantly improved remyelination and motor recovery (95)) and MSC transfected with neurotrophic factor (neuroprotective effect after optic nerve injury (96)).

8. GENETICALLY MODIFIED CELLS FOR TREATMENT OF OTHER NEUROLOGICAL DISEASES

Apart from the aforementioned neurological diseases, there are some pathological conditions for which stem cell based therapy as well holds a respectable promise. Among them, brain tumors and epilepsy are the most prominent. Stem cells transplanted into brain neoplasia exhibit natural tropism for tumor tissue. They are found near malignant cells far from the site of transplantation (97). This finding initiated the promising approach of using stem cells as vehicles which are able to deliver drugs to

destroy malignant cells. Moreover, this finding revived the concept of gene therapy of brain tumors which was faced with the almost unsolvable obstacle of successful gene delivery into CNS. In the last decade several approaches based on stem cell delivery have been tested and they included lytic viruses (98), prodrug-converting enzymes (99), immunomodulatory cytokines (100) and proteins with anti-angiogenic activity (101). Despite some optimistic reports from clinical trials describing prolonged life of the patients, improved protocols are needed to obtain more significant progress (reviewed by (102, 103)).

More than 30% of patients suffering from epilepsy do not have satisfactory therapeutic options (104). Cell transplantation therapy of such patients is based on the idea to transplant cells which will be instructed to produce molecules that exhibits anticonvulsant effects. So far, successful experiments in animal models with transplantation of genetically modified stem cells have been reported by using NSCs producing GABA (105) and embryonic/mesenchymal SC producing adenosine (106, 107).

9. CONCLUSION

Transplantation of stem cells as a strategy for the treatment of brain diseases has significantly evolved in the last decade. Exogenous embryonic or adult stem cells can be transduced to express a variety of genes and have been shown to promote functional recovery after transplantation into the lesioned brain, spinal cord or peripheral nerve. In addition, activation of endogenous stem cells apparently protect against inflammation, demyelination and neuronal degeneration. Translation of the first experiments on animals to the currently ongoing clinical trials has been achieved without major obstacles and the obtained results are promising. Further everyday progress in control of cell differentiation and improved protocols of cell transplantation are supporting a reasonable expectation: transplantation of genetically modified stem cells will in the following decades become a standard therapeutic procedure.

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