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Glial-restricted precursors as potential candidates for ALS cell-replacement therapy

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Amyotrophic lateral sclerosis is a multifactorial progressive neurodegenerative disorder leading to severe disability and death within 3-5 years after diagnosis. The main mechanisms underlying the disease progression are poorly known but according to the current knowledge, neuroinflammation is a key player in motor neurons damage. Astrocytes constitute an important cell population involved in neuroinflammatory reaction. Many studies confirmed their striking connection with motor neuron pathology and therefore they might be a target for the treatment of ALS. Cell-based therapy appears to be a promising strategy. Since direct replacement or restoring of motor neurons using various stem cells is challenging, enrichment of healthy donor-derived astrocytes appears to be a more realistic and beneficial approach. The effects of astrocytes have been examined using transplantation of glial-restricted precursors (GRPs) that represent one of the earliest precursors within the oligodendrocytic and astrocytic cell lineage. In this review, we focused on evidence-based data on astrocyte replacement transplantation therapy using GRPs in animal models of motor neuron diseases. The efficacy of GRPs engrafting is very encouraging. Furthermore, the lesson learned from application of lineage-restricted precursors in spinal cord injury (SCI) indicates that differentiation of GRPs into astrocytes before transplantation might be more advantageous in the context of axon regeneration. To sum up, the studies of glial-restricted precursors have made a step forward to ALS research and might bring breakthroughs to the field of ALS therapy in the future.

Key words: amyotrophic lateral sclerosis, glial-restricted precursors, astrocytes

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is an age-related, fatal, neurodegenerative disorder caused by selective and progressive loss of upper and lower motor neurons in the central nervous system (CNS). Various pathophysiological processes contribute to the motor neuron degeneration, namely: mitochondrial abnormalities, accumulation of intracellular aggregates, increased level of oxidized products, defects in axonal transport, glutamate excitotoxicity and glial cell pathology (Rothstein 2009). The main clinical consequence of underlying ALS pathophysiology is gradual muscle weakness and, at the end stage of the disease, respiratory failure leading to death (Gordon 2013). The ALS prevalence in Europe is 2.16 per 100 000 person per year. The incidence rate is higher among men (3.0 per 100 000 person/year) than women (2.4 per 100 000 person/year) (Logroscino et al. 2010). The average age of the disease onset is between 50 and 60 years (Rothstein 2009) and the patient survival in majority of cases is less than 3 years after occurrence of symptom onset (Al-Chalabi and Hardiman 2013). The vast majority of ALS cases (nearly 90%) are sporadic of unknown etiology (Kiernan et al. 2011). The remaining ALS cases are familial, and of those 20% are associated with the mutation in the Cu/Zn superoxide dismutase (SOD1) gene (Rosen et al. 1993). Mutations in 43-kDa TAR DNA-binding protein (TARDP) gene (Kabashi et al. 2008), fused/translocated in liposarcoma (FUS) gene (Kwiatkowski et al. 2009) are responsible for 5% familial ALS cases each. The several other pathogenic mutations associated with inherited ALS have been examined as well (Ticozzi et al. 2011). Although the causes of sporadic ALS are yet not fully

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understood, few possible factors contributing to the onset of the disease have been suggested, such as genetic predispositions, advanced age, smoking and athleticism (Gordon 2013). Amyotrophic lateral sclerosis is incurable, lethal disease. Despite many clinical trials aimed at neuroprotective drug discovery, so far there is only one approved medication – riluzole, that slows the progression of the disease (Bensimon et al. 1994).

Numerous approaches have been made in order to identify an effective therapy for ALS. The promising approach constitutes stem-cell replacement therapy. Many studies have been done to replace dead or damaged motor neurons using various stem cells such as induced pluripotent stem cells (iPS cells) (Dimos et al. 2008), embryonic stem cells (ESCs) (Li et al. 2005, Lee et al. 2007), multipotent spinal cord neural stem cells (NSCs) (MacDonald et al. 2003). Although studies confirmed survival of transplanted motoneurons derived from ESCs, this approach is extremely challenging due to unfavourable environment of motor neurons (MNs) in ALS patients and difficulties in integration of engrafted neurons (Lee et al. 2007). Other studies revealed that bone marrow and mesenchymal stem cells (MSCs) derived from bone marrow provide neurotrophic and neuroprotective effects in ALS mouse models (Pastor et al. 2012) and in patients suffering from ALS (Karussis et al. 2010). Similarly, the engraftment of adult hematopoietic stem cells (HSCs) into spinal cord of muscle deficient (mdf) mice contributes to the improvement of motor functions through production of glial-derived neurotrophic factor (GDNF) (Cabanes et al. 2007). Thus, transplanted stem cells in animals and ALS patients are likely beneficial due to their supportive influence on degenerating neurons and increased production of growth factors. All these findings highlight that non-neuronal cells replacement is a promising strategy for ALS treatment. In order to target specific cell population which might be effective, the mechanisms underlying motor neurons degeneration have to be taken into consideration. Recent evidence indicates that neuroinflammation is one of the key mechanisms underlying neurodegeneration in ALS. The cell populations involved in the neuroinflammatory reaction are microglia, astrocytes and T-cells (Philips and Robberecht 2011).

Astrocytes represent the largest cell population in the CNS serving under normal conditions as a structural, metabolic and trophic support to motor neurons. However, in animal models of ALS, astrocytes undergo dramatic morphological and functional changes and become "neurodegenerative cells" in spite of their neuroprotective nature (Markiewicz and Lukomska 2006). The reactive astrocytes demonstrate a diminished expression of glutamate transporter (GLT-1) that alters the capacity of glutamate transport and leads to exitotoxicity. Furthermore, the oxidative stress and peroxynitrite generated in reactive astrocytes contribute to the defective glutamate homeostasis that causes motor neurons degeneration (Barbeito et al. 2004). Many studies confirmed the toxic influence of astrocytes on motor neurons. Co-cultures of motor neurons and mutated SOD1 overexpressing astrocytes revealed that these astrocytes are toxic to MNs, likely leading to their degeneration (Nagai et al. 2007, Marchetto et al. 2008). The *in vivo* studies confirmed that $SOD1^{G93A}$ glial-restricted precursors capable of generating astrocytes contribute to motor neuron degeneration (Papadeas et al. 2011). Moreover, Haidet-Phillips and coauthors (2011) indicate that human astrocytes generated from *post-mortem* tissues of sporadic and familial ALS patients were similarly toxic to cultured motor neurons. These data provide evidence underscoring potential role of astrocytes in ALS cell-replacement therapy. The effects of astrocytes can be examined using transplantation of lineage-restricted precursors such as glial-restricted precursors as an alternative to stem-cell based therapies.

GLIAL-RESTRICTED PRECURSORS

Lineage-restricted precursors have recently become a promising strategy for CNS transplantation. Such cells include neuronal-restricted (NRPs) and glial-restricted precursors (GRPs), both having limited differentiation potential (Rao 1999). GRP cells derive from multipotent neuroepithelial stem cells (NEP) and represent one of the earliest precursors within the oligodendrocytic and astrocytic cell lineage. Their identification is based on the presence of the surface marker A2B5 that distinguishes them from NRPs expressing E-NCAM (Rao and Mayer-Proschel 1997). GRPs isolated from the embryonic spinal cord are tripotential and can generate two phenotypic classes of astrocytes and oligodendrocyte progenitors (Rao et al. 1998) (Fig. 1). The oligodendrocyte progenitors known as "O-2A progenitors", which were initially isolated

from the rat optic nerve, are the best defined derivative of GRPs widely distributed within grey and white matter of the CNS (Raff et al. 1983). They were considered as bipotential cells by their ability to differentiate *in vitro* into both oligodendrocytes and type-2 astrocytes (Raff et al. 1983, Gregori et al. 2002). The term "O-2A progenitors" has been disappearing from the general use and is being replaced by "oligodendrocyte precursor cells (OPCs)" or "NG^{2+} cells". OPCs express various molecular markers including chondroitin sulphate proteoglycan neuron-glial antigen 2 (NG2) and the platelet-derived growth factor receptor type α (PDGFRα) (Nishiyama et al. 1996). Recent studies suggest that these cells might have wider differentiation potential *in vitro* and, aside from glial cell generation, might be also able to produce neurons (Kondo and Raff 2000, Sypecka and Sarnowska 2013, Sypecka et al. 2013). Surprisingly, OPCs have an ability to develop into Schwann cells capable of remyelinating axons during CNS repair (Zawadzka et al. 2010). Due to the capability of OPCs to generate cells involved in remyelination, they are currently being studied as a potential source for cellular therapies in demyelinating disorders such as multiple sclerosis (Wang et al. 2011, Kim et al. 2012) or spinal cord injury (Keirstead et al. 2005, Sharp et al. 2010). Although OPCs *in vitro* have wide differentiation potential, *in vivo* in healthy brain and spinal cord they give rise only to myelinating oli-

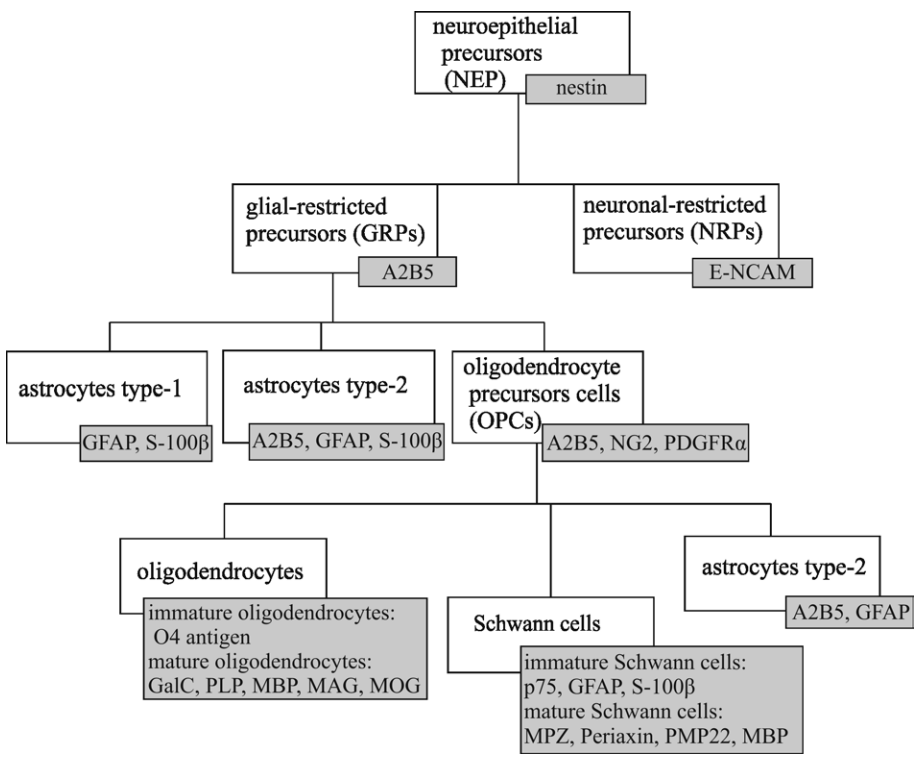

Fig. 1. Schematic representation of the lineage relationships with panel of markers expressed by each cell. Neuroepithelial precursors (NEP) expressing nestin can generate glial-restricted precursors (GRPs) and neuronal-restricted precursors (NRPs) expressing E-NCAM (embryonic form of neural cell surface molecule). GRPs cells can give rise to oligodendrocyte precursor cells (OPCs) with characteristic markers such as A2B5 (cell surface ganglioside epitope expressed on GRPs), NG2 (chondroitin sulphate proteoglycan neuron-glial antigen 2), PDGFRα (platelet-derived growth factor receptor type α). GRPs are also the ancestor of astrocytes type-1 (which may have common antigenic features with astrocytes generated by exposure of GRPs to bone morphogenetic protein 4 – BMP-4) and astrocytes type-2 (which may have common antigenic features with astrocytes generated by exposure of GRPs to ciliary neurotrophic factor – CNTF). OPCs (oligodendrocyte precursor cells) in turn can give rise to both lineages: oligodendrocytes and astrocytes. Recent studies (Zawadzka et al. 2010) indicate that OPCs might also generate Schwann cells when exposed to appropriate environmental conditions. Abbreviations: (GFAP) glial fibrillary acidic protein; (GalC) galactocerebroside; (MAG) myelin-associated glycoprotein; (MBP) myelin basic protein; (MOG) myelin oligodendrocyte glycoprotein; (p75) low-affinity nerve growth factor receptor; (PLP) proteolipid protein; (S-100β) β subunit of a calcium binding protein; (MPZ) myelin protein zero; (PMP22) peripheral myelin protein 22.

godendrocytes. Moreover, despite their enhanced proliferation resulting from the increased spinal cord neurodegeneration in a mouse model of ALS, they are also committed to the oligodendrocyte lineage (Kang et al. 2010). Thus, promising candidates for ALS therapy include only glial-restricted precursors and astrocytes derived from them.

GLIAL-RESTRICTED PRECURSORS AND GRPs-DERIVED ASTROCYTES AS PROMISING CANDIDATES FOR ALS AND SCI THERAPY

Many studies aimed at verifying glial-restricted precursors (GRP) usefulness in CNS disorders have been conducted in recent years. Some of these studies confirmed that, in the opposition to fetal multipotent neuroepithelial (NEP) stem cells both types of lineage restricted precursors, NRPs and GRPs, survive excellently, differentiate robustly and migrate selectively after engraftment into intact and injured CNS (Lepore et al. 2004, Lepore and Fisher 2005). Thus, it appears that the mixture of these lineage restricted precursors constitutes an optimal therapeutic strategy. Transplantation of glial-restricted progenitors alone have been studied in animal models of demyelinating diseases such as multiple sclerosis (MS) (Totoiu et al. 2003, Perez-Bouza et al. 2005) and spinal cord injury (SCI) (Han et al. 2004). The results of these studies indicate that GRPs differentiated into both myelinating oligodendrocytes and astrocytes, migrated along the white matter (Han et al. 2004, Perez-Bouza et al. 2005) and consequently contributed to myelin repair and improvement of locomotor function (Totoiu et al. 2003).

While there is a substantial progress in myelin repair through transplantation of cells capable of generating oligodendrocytes, little is known about astrocytes and their application in therapy of CNS disorders. Despite the fact that astrocyte dysfunction underlies many of CNS disorders, there is a slow progress in astrocyte transplantation. Technically, transplantation of astrocytes does not appear to be as challenging as motor neurons engraftment in ALS therapy (Rizzo et al. 2013). Now a query arises, if engrafted healthy astrocytes could be negatively influenced by the pathologic environment of ALS and turn into "neurodegenerative" astrocytes. Optimistically, initial research revealed that astrocytic precursors might demonstrate some resistance to ongoing pathological process (Vargas et al. 2005).

Recently, many studies validating the role of astrocyte replacement using GRPs as a novel neuroprotective therapy in animal models of motor neuron diseases have been conducted. Lepore and colleagues (2008) initially demonstrated that transplantation of rodent-derived GRPs in order to enrich normal astrocytes in $SOD1^{G93A}$ rat promoted protection of motor neurons and delayed disease progression. This work indicates that transplanted GRPs have an influence on the levels of primary glutamate transporter (GLT-1) leading to neuroprotection. However, authors excluded that enhanced secretion of some neurotrophic factors might have biased their study. Analogous research has been made recently with GRPs derived from human fetal forebrain (Lepore et al. 2011). Transplanted hGRPs into $SOD1^{G93A}$ mice showed robust survival and migration along both grey and white matter. Nevertheless, it did not improve motor neuron protection and did not bring any therapeutic effects. Investigation of the fate of transplanted hGRPs revealed that at disease end-stage approximately 50–80% of hGRPs remained committed to astrocytes. Although the majority of GRPs generated the desired cell type, the differentiation into oligodendrocytes was also observed (Lepore et al. 2011). This finding suggests that the transplantation of more differentiated cells with desired phenotype might offer greater advantages in terms of functional recovery. It might be beneficial in terms of glutamate transporter (GLT-1) delivery since it was demonstrated that differentiation of oligodendrocytes from GRPs leads to downregulation of all glutamate transporter subtypes (Maragakis et al. 2005).

Some studies revealed that more differentiated population of astrocytes derived from GRPs (GRPs-derived astrocytes – GDAs) might be a better cell source for transplantation into spinal cord injury (SCI) than undifferentiated GRPs. Davies and coworkers (2006) transplanted GDAs derived from embryonic glial precursors into acute adult rat SCI. In order to obtain GDAs before transplantation, they exposed GRPs to bone morphogenetic protein-4 (BMP-4) that resulted in generation of cells with a phenotype of astrocytes type-1 ($GDAs^{BMP}$). Engraftment of these cells promoted axon regeneration leading to functional recovery. However, $GDAs^{CNTF}$ generated by the exposure to the ciliary neurotrophic factor (CNTF) did not

provide such benefits and, in addition, caused neuropathic pain syndromes persisting for 5 weeks after injury (Davies et al. 2008). This finding demonstrates that the transplantation of wrong cells into diseased animal not only can fail to provide axonal regeneration but also might be harmful to the recipient. Similar beneficial effects that were obtained after transplantation of $GDAs^{BMP}$, were also observed with human glial-derived astrocytes. Transplantation of $hGDAs^{BMP}$ obtained through differentiation of GRPs derived from fetal nervous tissue into the spinal cord lesion of rats resulted in prolonged survival and intense migration of grafted cells (Davies et al. 2011, Jin et al. 2011) that leaded to modest (Jin et al. 2011) or significant (Davies et al. 2011) functional recovery. Similarly to rat CNTF-induced astrocyte populations, human $GDAs^{CNTF}$ failed to provide locomotor improvement (Davies et al. 2011). The benefits provided after transplantation of specific cell type such as $GDAs^{BMP}$ are not coincidental. The mechanisms by which transplanted cell population contribute to the improvement of CNS injury site are not well-known. However, previous studies revealing the expression of brain-derived neurotrophic factor (BDNF) in $GDAs^{BMP}$ indicated that this neurotrophic factor might contribute to rescue of neurons (reviewed by Noble et al. 2011). In contrast to Jin and coworkers (2011) and Davies and others (2011), recent studies of Haas and Fischer (2013) have not revealed the differences in promoting the axonal regeneration between both groups of GDAs ($GDAs^{BMP}$ and $GDAs^{CNTF}$). Since the transplantation of GDAs has been advantageous in spinal cord injury, the question emerges, if such astrocytes precursors transplanted in an ALS environment are also able to provide more benefits than undifferentiated GRPs.

FUTURE PERSPECTIVES

Taking into consideration that the more differentiated glial-restricted precursors towards astrocytes (GDAs) have brought more benefits than undifferentiated GRP in the context of SCI, this manipulation may represent future direction for non-neuronal cells replacement therapy of ALS. Obviously, it will increase the yield of desired cell type before transplantation and thus may result in greater axonal improvement. Further studies should also take into account the appropriate "dosage" of cells for effective outcomes and also the maximal "dosage" of cells that will still be well-tolerated (Lepore et al. 2011). Delivering cells into many transplant regions appears to result in better efficacy and therefore constitutes a promising future strategy (Xu et al. 2011).

Another approach is to generate autologous cells for transplantation that may circumvent the necessity of a long-term immunosuppressive therapy and would prevent graft rejection. Consequently, it may lead to better survival and migration patterns of such cells. Allogeneic transplants even into immune privileged regions of CNS need a long-term immunosuppression regimen. Optimal immunosuppressive therapy contributes to the survival and efficacy of grafted cells (Yan et al. 2006). As an alternative for allogeneic cells, autologous induced pluripotent cells (iPS cells) might circumvent the need for immunosuppressive therapy. Patient-specific induced pluripotent stem cells are generated by reverting somatic cells to a pluripotent state and therefore they are able to adopt to various phenotypes (Kiskinis and Eggan 2010). Preclinical studies are currently being held at Johns Hopkins University that involve human induced pluripotent stem cell-derived glial-restricted precursors (iPS-GRPs). These studies may represent the initial progress of a viable autologous cell therapy for ALS patients. The aim of this research is to indicate whether iPS-GRPs from ALS patients will be healthy with neuroprotective capacities or whether these cells will demonstrate pathologic ALS-specific features. Other concern is related to the possibility of uncontrolled cell growth after transplantation or decreased survival of cells derived from ALS-patient (Maragakis et al. unpublished data).

Another approach to avoid immunosuppression might be a production of autologous embryonic stem cells (ESCs) by nuclear transfer. Furthermore, glial-restricted precursors from ESCs not only can serve in the classical cell replacement therapy, but also, after genetic modification they might be used to deliver therapeutically active neurotrophic factors. Many growth factors are capable of rescuing the motoneuron cell bodies in the spinal cord (reviewed by Henriques et al. 2010). Although pluripotent stem cells such as embryonic stem cells provide an attractive approach for regenerative medicine, there is a possible risk of tumorigenecity. In order to predict the possibility of tumor formation, recent studies have developed a comparative analysis of the efficiency of tumor genesis after various ESCs engraftments (Gordeeva and Nikonova 2013).

All studies mentioned in this review provide promising directions in which future research would evolve in order to find an effective cell replacement therapy for ALS. It should be underlined that there is still a strong need of further evaluation of GRPs potential in ALS animal models before it will be translated into clinic. We should also keep in mind that cell-based therapy will not lead to a cure of the disease but might help restore some lost CNS functions. Furthermore, transplantation of cells should be repeated since cell replacement is a long-term medicine.

CONCLUSIONS

The major pathogenic mechanisms leading to the selective and progressive degeneration of motor neurons during amyotrophic lateral sclerosis are still poorly understood and therefore the effective treatment or cure for ALS has not yet been developed. Recent development of stem cell approaches has highlighted their usefulness in treatment of neurodegenerative disorders.

Considering that the replacement of damaged motor neurons is difficult to achieve, cell-based therapy should be focused on non-neuronal cells in the context of ALS treatment. Glial cells are crucial participants in neuroinflammation and ALS progression and therefore their replacement might be neuroprotective. In this review, the great potential of glial- restricted precursors and astrocytes derived from them in the axonal regeneration has been underscored. The data described suggest that the transplantation of astrocytes derived from glial-restricted precursors (GDAs) could be even more advantageous than undifferentiated GRPs in the context of both spinal cord injury and degeneration of motor neurons. Further research should involve the engraftments of desired cell types into multiple regions to reduce ALS symptoms. Finally, future studies should include generation of autologous glial-restricted progenitors in order to prevent immunosuppression and graft rejection.

Taken together, the studies of glial-restricted precursors have made step forward to ALS research and might bring breakthroughs to the field of ALS therapy in the future.

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