

Searching for oligodendrocyte precursors for cell replacement therapies

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Inherited or acquired oligodendrocyte deficiency or abnormalities usually lead to severe neurological disorders, common in humans and animals. The wide spectrum of diseases to be treated, their frequency and lack of effective treatments result in an urgent need to elaborate alternative options such as cell replacement therapies. Elaborating the protocols for the efficient oligodendrocyte progenitor cell (OPC) generation for neurorestrative purposes still meets the essential obstacles. Searching for the most convenient, accessible and rich sources of stem-like cells is the first of them. The immunological barrier is another problem, which could however be solved by usage of either the allo- or the autografts and the pure, xeno-free compounds for the cell propagation. The third criterion is the efficiency of the progenitor derivation, proliferation and purification. The effective cellular replacement experiments carried on animal models of congenital and acquired neurodegenerative disease approached their translation into clinical practice. Notwithstanding due to urgent need for treatment of a broad spectrum of traumas and neurodegenerative disorders accompanied by hypo/demyelination, different cell sources and alternative strategies should necessarily be tested to expand the treatment options.

Key words: cell-based therapies, leukodystrophies, myelination, neural stem cells, oligodendrocyte

INTRODUCTION

The NG2 proteoglycan-expressing cells are the oligodendrocyte precursors that terminally differentiated are capable of myelinating the central nervous system (CNS). Inherited or acquired oligodendrocyte deficiency or abnormalities usually lead to severe neurological disorders, common in humans and animals. The wide spectrum of diseases to be treated and their frequency result in an urgent need to elaborate the alternative cell replacement therapies. Transplanting oligodendrocyte progenitors offers multiple advantages. They include supplying oligodendrocytes resulting in at least partial remyelination, attenuating local inflammatory processes by neurotrophins secreted by grafted progenitors and mobilizing endogenous neural stem cells/progenitors for neuroreparative purposes.

The precursor cells could be either isolated from the

tissues or generated *in vitro* from stem cells. The cell isolation, propagation and *in vitro* maintenance basically imitate their *in vivo* development.

OLIGODENDROCYTE DEVELOPMENT

Oligodendrocyte progenitors are derived from the restricted area of the ventral neuroepithelium. In the developing neural tube, the notochord and the floor plate express the opposing gradients of morphogenic signals and establish the domains from where different neural cell lineages are generated (Bongarzone 2002). The commitment of neural stem cells to glial lineage depends on the Sonic Hedgehog (Shh) concentration and expression of several both transcriptional (e.g., Olig1, Olig2, Sox10) and trophic factors (e.g., PDGF). After the developmental fate is determined, the precursors migrate, sometimes over considerable distances, and populate the rest of the self-forming CNS. A number of extracellular matrix (ECM) molecules play an instructive role in the control of migration, for instance the glycoprotein Tenascin-C (Garwood et al. 2004) or metalloproteinases MMP-9 (Uhm et al. 1998,

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Larsen et al. 2006) and MMP-12 (Larsen and Yong, 2004). The response to extracellular signals, which plays a crucial role both in the cell proliferation and migration, is possible due to an array of integrin receptors (Baron et al. 2002, Watkins and Barres 2002). Once they reach their final destination, progenitors stop to divide and differentiate into mature, myelin-forming oligodendrocytes. *In vivo*, the process is known to depend on the concentration of biological factors like thyroid hormones (Fernandez et al. 2004, Schoonover et al. 2004) and IGF-1. *In vitro*, numerous mitogens and trophic factors promote oligodendrocyte precursors proliferation, maturation and survival: PDGF, bFGF, triiodothyronine, progesterone, insulin, transferrin, and many others.

In response to microenvironmental signals, the differentiating progenitors change their morphology from small bipolar cells to few process-bearing immature oligodendrocytes (Fig. 1A-C). Maturation is characterised by an increase in the number of processes, as well as in their length and complexity (Fig. 1D-F). The morphological changes and initiation of myelin genes expression is accompanied by a transient change in the antigen profile: A2B5 and NG2 markers are linked to precursors, the anti-O4 antibody stains immature oligodendrocytes. Myelinating, mature cells express sequentially GalC, PLP, MBP, MAG and finally MOG (Grinspan 2002).

ISOLATION OF OLIGODENDROCYTE PRECURSORS FROM TISSUES

The A2B5/NG2-positive precursors could be isolated from CNS and further propagated and differentiated *in vitro* in the purpose of disease modeling, pharmaceutical screening and cell replacement therapies. There are a few procedures commonly used for oligodendrocyte progenitor isolation from the CNS tissues. The classical method based on different adhesive properties of particular neural cell types, originally described by McCarthy and deVellis (1980), is commonly used with some minor modifications (Gu et al. 1997, Jana et al. 2007, Sypecka et al. 2009a). Preparation of mixed glial primary culture is the initial step, followed by the 10-14 DIV culturing and then sequential mechanical dislodging the microglia, then the oligodendroglia, while astrocytes remain attached to the plastic.

Another procedure is based on the immunopanning technique, employed along with different oligodendro-

cyte specific antibodies: A2B5, CNP, O4 (Trotter and Schachner 1989, Back et al. 1998, Ben-Hur et al. 1998, Cahoy et al. 2008, Ogawa et al. 2011). The mechanical detachment of the bound cells could be applied (Gard and Pfeiffer 1993, Warrington et al. 1992) but usually the cells attached to micro-beads are positively selected by sorting. The protocols typically involve magnetic-associated cell sorting: e.g., purification on secondary antibody-conjugated magnetic microbeads followed by separation on a ferromagnetic column (MACS) (Shankar et al. 2003, Windrem et al. 2004, Cizkova et al. 2009, Letzen et al. 2010) or fluorescence-activated cell-sorting in flow cytometer (FACS) (Maric et al. 2003, Nielsen et al. 2005, Assanah et al. 2006).

Since NG2 cells meant for neuroreparative therapies are restricted to CNS, generating oligodendrocyte progenitors from stem cells is widely discussed and it seems to be an inevitable necessity.

GENERATION OF OLIGODENDROCYTE PRECURSORS *IN VITRO*

Cell sources

Elaborating the protocols for the efficient OPC generation for neurorestorative purposes meets with major obstacles. Searching for the most convenient and accessible cell source is the first of them. The prevailing part of studies has been performed on the human embryonic stem cells (Zhang et al. 2006, Izrael et al. 2007, Kerr et al. 2010, Letzen et al. 2010, Sundberg et al. 2010) or immortalized neural stem cell lines (Neri et al. 2010, Salazar et al. 2010). The exploitation of certain potential cell sources-like aborted fetuses for instances (Poltavtseva et al. 2002, Liang et al. 2006, Amarilglio et al. 2009, Salazar et al. 2010, Sandrock et al. 2010) would presumably be seriously limited due to both ethical and legislative controversies. Notwithstanding, basically any of the known stem cells reservoirs-like adipose tissue, bone marrow, dental pulp, Wharton's jelly *et cetera*-might be used for derivating oligodendrocyte progenitors.

Among them, the human umbilical cord blood seems to offer the practical advantages (Tracy et al. 2008, Chua et al. 2009, Ali and Bahbahani, 2010, Jablonska et al. 2010, Luo et al. 2010). It is a feasible source due to numerous public banks of cord blood and a possibility arises to use it for autologous transplantations. We have proposed the human umbilical cord

blood-neural stem cell (HUCB-NSC) line as a very convenient and ethically uncontroversial source of oligodendrocytes for pharmaceutical screening and cellular replacement therapies (Buzanska et al. 2009, Sypecka et al. 2009b). This line of cells which has

already been neurally-biased, untransformed and proliferating well is a potential alternative for human embryonic stem cell lines.

In recent years, induced pluripotent stem cells (iPSCs) have been generated, offering a new future

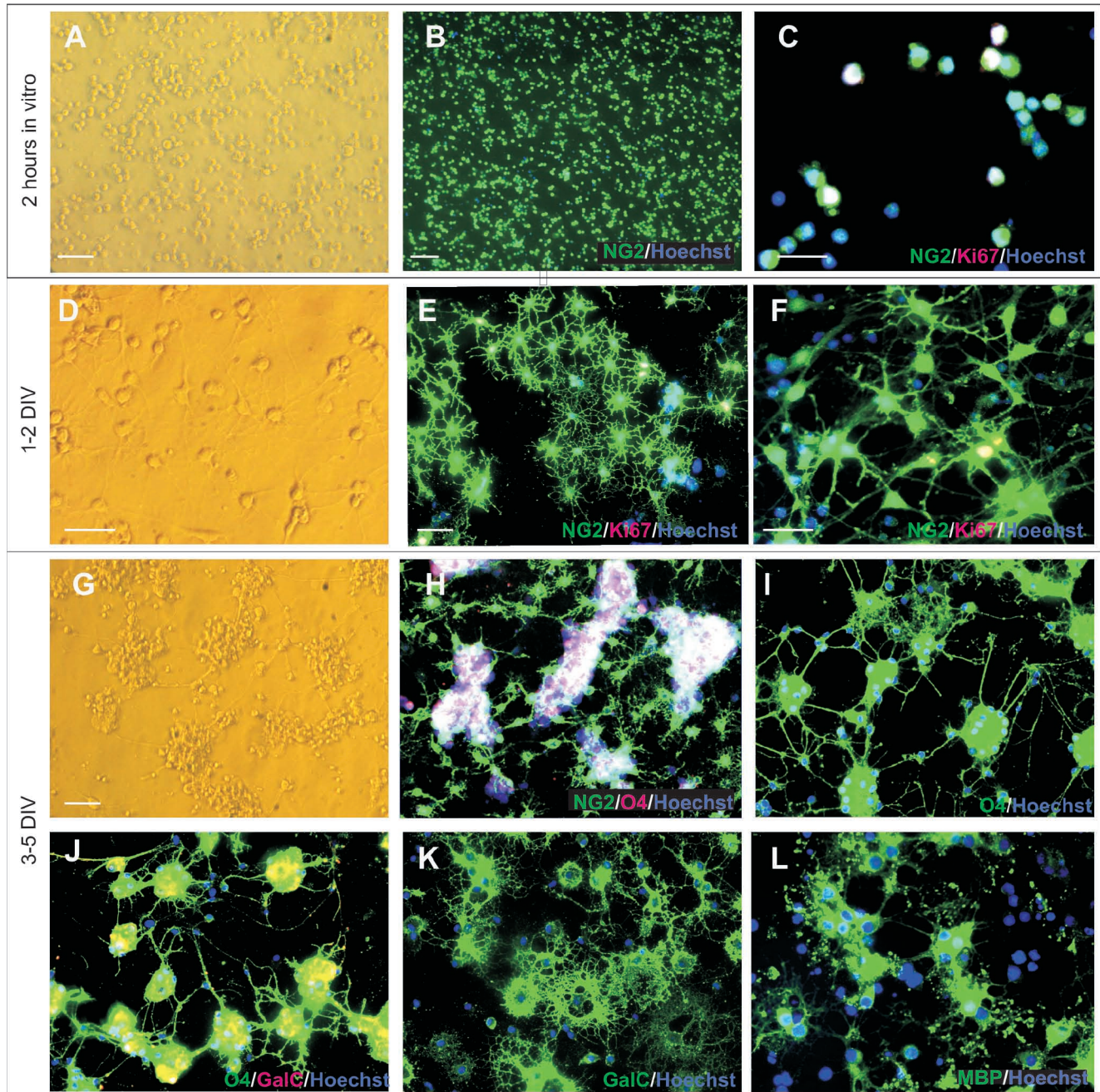


Fig. 1 Oligodendrocyte precursor differentiation and maturation in vitro: two hours after seeding (A-C), during first and second DIV (D-F) and from 3rd to 5th DIV (G-L). A-C: small round NG2-positive (B, C) and Ki-67-expressing proliferating progenitors (C); D-F: few process-bearing developing and proliferating (F) NG2 cells: an considerable increase in the process length and their complexity is clearly visible; G-L: differentiating oligodendrocytes express O4 antigen (H, I, J), then GalC marker (J, K) and finally the myelin protein MBP (L). The cell culturing for the additional couple days results in the increase in number of mature, MBP⁺ oligodendrocytes.

potentiality of autologous grafting. The iPSCs are the product of somatic (usually dermal fibroblasts) factor-based cell reprogramming into an undifferentiated embryonic-like state and therefore might adopt various phenotypes (Kim et al. 2009, Boué et al. 2010, Li et al. 2010). The feasibility to collect tissue would enable the research workers to prepare the patient-targeted therapy. The first reports of generating neural progenitors from iPSCs and differentiating them into neurons and glia (including oligodendrocytes) in both *in vitro* studies and in animal models of spinal cord injury (SCI) have recently been published (Tokumoto et al. 2010, Tsuji et al. 2010, Ogawa et al. 2011). The very recent reports suggest however that the cell dedifferentiation into iPSCs might even be omitted by the direct cell conversion into desired phenotypes (Szabo et al. 2010, Vierbuchen et al. 2010), e.g., the fibroblast have been shown to be reprogrammed into neurons (iN). This could be another alternative for the future patient-specific cell therapies.

Actually, each of the known reservoirs of stem cells could be considered for the cell-based therapies as far as the protocols for efficient oligodendrocyte derivation would be elaborated, obeying some crucial indications.

Quality of grafts-a risk of contamination

The question of the immunological barrier is the second problem to be solved while preparing methods of OPC propagation. Almost all of the known protocols involve animal-derived factors, like bovine/horse serum applied in relatively high doses (1-10%), digesting enzymes, trophic factors, extracellular matrix components used to improve cell adhesion and differentiation, Matrigel (a mixture of extracellular matrices, proteoglycans, and growth factors), feeder cells etc. (e.g., Hermann et al. 2004, Jang et al. 2010, Sharp et al. 2010). Some of them are still undefined (like serum for instance) and may have unknown effects on the cell characteristics and differentiation. The animal-derived factors are likely to contaminate cell grafts and consequently trigger immunological response. Therefore procedures of clinical therapies elaboration should be obligatorily based on defined conditions and the pure, xeno-free compounds (Nagaoka et al. 2010, Rajala et al. 2010). Some procedures aimed at generating nearly pure oligodendrocyte population are based on neuroblastoma-conditioned medium (Broughton et

al. 2007, Fu et al. 2007, Kennea et al. 2009), which might be hazardous to patients.

Another type of risk is associated with the usage of the approved stem cell lines that are cultured and passaged *in vitro* for the prolonged time and therefore develop genetic dysfunctions due to their age. Long-time culture was shown to affect mitochondrial function which is associated with cellular metabolism and their potential to adversely affect ROS generation and integrity of mtDNA (Xie et al. 2011). As they proliferate, genetic abnormalities often develop and resulting ramifications might contribute to tumor formation (Artandi and DePinho 2010). In the view of these objectives, the natural reservoirs of stem cells like either cord blood or patient-derived tissues (adipose, fibroblasts) seem to be a fine alternative for elaborating treatment options.

Quantitative aspect

The third criterion is the efficiency of progenitor derivation and propagation. The usage of pluripotent stem cells (e.g., embryonic, mesenchymal) requires generating neural stem cells in the initial step and promoting gliogenesis in the following. The currently available methods are usually several weeks long and are based on sequential application of relatively high doses of several tropic factors and neuromorphogens (e.g., Hu et al. 2009, Sundberg et al. 2010). These requirements make them expensive and time-consuming and in consequence unfortunately might limit their common therapeutic application. The efforts therefore should be concentrated on the selecting the most potent factors promoting oligodendrogenesis, allowing to shorten the multistep *in vitro* protocols. An alternative - the clinical application of uncommitted ESC - is associated with the health hazard such as malignant transformation, resulting even in multifocal tumor formation (Amarilgio et al. 2009).

FUTURE PERSPECTIVES

The effective cellular replacement experiments carried on animal models of congenital and acquired neurodegenerative disease approached their translation into clinical practice (e.g., Windrem et al. 2004, Neri et al. 2010). Actually, the first Phase I of clinical trial with HuCNS-SC to treat congenital dysmyelinating Pelizaeus-Merzbacher disease (PMD) has begun in February 2010 (StemCell, Inc). In PMD, a defective

PLP gene on the X chromosome leads to insufficient myelination of axons in the brain, neurological impairment and eventually death in the fatal forms of the disease. The cell transplantation-based strategies might be the only potential effective treatments for leukodystrophies resulting from genetic defects.

Another Phase I multi-center trial is planned by Geron and is designed to assess the safety and tolerability of GRNOPC1 (hESC - Derived Therapies) in patients with American Spinal Injury Association (ASIA) Impairment Scale grade A subacute thoracic spinal cord injuries. Transplantation of bone marrow stromal cell aimed at treatment for acute spinal cord injury (SCI) have been already applied by several clinics (e.g., Kansai Medical University Moriguchi, Osaka, Japan; Cairo University School of Medicine Cairo, Egypt; Sita Bhateja Speciality Hospital Bangalore, Karnataka, India). Therapies based on the autologous bone marrow transplantation (Sykova et al. 2006, Geffner et al. 2008, Deda et al. 2009), as well as the cells derived from human umbilical cord blood (Kang et al. 2005) have been reported to efficiently contribute to the functional improvement. Currently, there is another announcement about the patient recruitment for the clinical trial of umbilical cord blood cell grafting into SCI, planned by researchers from Chinese University of Hong Kong. The other very promising project is based on the transplantation of autologous olfactory ensheathing cells for treatment of complete human spinal cord injuries- a Phase I clinical trial is about to be started in Wroclaw Medical University, Poland (www.ClinicalTrials.gov).

The usage of oligodendrocyte progenitors in cell replacement therapies offers multiple advantages. On one hand, the application of glia-committed cells ensures their differentiation into myelin forming oligodendrocytes thus efficiently contributing to CNS remyelination. On the other hand, there is an ever growing list of evidences that NG2 cells actually possess an intrinsic neurogenic potential and they are capable of neuronal differentiation in response to environmental stimuli (Belachew et al. 2003, Aguirre and Gallo 2004, Sypecka et al. 2009a), holding out hope of repairing a damaged tissue. The attenuation of local inflammatory processes, mobilization of endogenous stem cells and the trophic support provided by the progenitors are the additional benefits of the cell replacement, promoting neurorestoration (Lee et al. 2007; Aharonowiz et al. 2008; Einstein et al. 2009).

CONCLUSION

Taken together, there is an enormous, hope-raising progress in oligodendrocyte replacement strategies, resulting in spectacular effect of clinical trials on diseases evoked by myelin disorders. The cell reservoir used for glial precursor derivation has been significantly enlarged by adding the well-recognized stem cell sources, as well as by the new techniques of the adult, tissue-specific cells differentiation either into iPSc or by direct reprogramming into desired phenotypes. Numerous methods aimed at promoting oligodendroglialogenesis have been established. This creates the future possibilities of generating oligodendroglial precursor and future elaborating the autologous patient-targeted therapies. Notwithstanding, due to urgent need for treatment of a broad spectrum of traumas and neurodegenerative disorders, different cell sources and alternative strategies should necessarily be tested to expand treatment options.

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