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CNS Regenerative Medicine and Stem Cells

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Abstract. Stem cells research has passed a long and exciting way from discovery to clinical applications. Every year more and more scientific reports and solid research breakthroughs are published in this fascinating field making it difficult to follow new discoveries and cover the history. In the current review we overviewed the history of stem cells research starting from the discovery and ending with the current state-of-art. We discussed the obstacles and future perspectives of the cell-based therapy, with a special focus made on the protection and regeneration of the lost functions after injury/degeneration of adult central nervous system.

Keywords: Stem cell; Embryonic stem cell; Induced pluripotent stem cell; Mesenchymal stromal cell; Cell therapy; CNS regeneration; ALS

Introduction

"Omnis cellula e cellula"
Rudolph Virchov

Stem cell research field gained an increased interest during the past two decades and became highly recognized not only among professionals, but also in public. Two Nobel Prizes in Physiology or Medicine in 2007 and 2012 were awarded for the research in stem cell field. The reasons for this are multiple. First of all, stem cells (SCs) present new perspectives in cell-based therapies and regenerative medicine, therefore bringing new hopes for patients with incurable conditions. Secondly, SCs isolated from patients can serve as an in vitro model of various diseases and drug development. Finally, understanding the biology of SCs will improve our knowledge of embryogenesis and cell biology in general.

Definition and properties of SCs

Stem cell is an undifferentiated, karyotypically normal cell with the capacity to self-renew and ability to generate differentiated cells. The most important criterion of stemness is called self-renewal, that is the ability to generate at least one identical copy of the daughter cell. The ability of cell to differentiate into other cell types is the cell potency. According to this characteristic SCs can be totipotent, pluripotent and multipotent. Totipotent are cells that can give rise to all cell types, including cells of the trophectoderm lineage. In mammals only zygote and early blastomeres are totipotent. Pluripotent cells can generate the cells of all three germ layers as well as germline, but not the extraembryonic trophoblast. Multipotent cells can give rise to a restricted subset of tissue-specific cell types (within one germ layer). The SC hierarchy is schematically described in Fig.1. SCs are classified by their source and the tissue they are typically generated from, as well as the stage during which they appear in the lifetime of the organism: 1) embryonic stem cells (ESC) - pluripotent and give rise to all tissues in an organism; 2) somatic (adult-derived) stem cells - multipotent and found in different tissues in the fully developed organism and in umbilical cord blood; and 3) induced pluripotent stem (iPS) cells-capable of regaining their pluripotent properties after the artificial introduction of transcriptional factors into the somatic cell (Takahashi and Yamanaka 2006). They are able to differentiate to diverse specialized cell types, including neuronal and glial cell lineages (Nistor, Totoiu et al. 2005; Lee, Shamy et al. 2007).

Embryonic stem cells

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation in vitro (Evans and Kaufman 1981; Martin 1981), while still keeping the potential to generate a diverse range of functional precursors and terminally differentiated cells. ESCs are derived from the inner cell mass (ICM) of the pre-implantation blastocyst (Fig. SC hierarchy). Mouse ESCs (mES) cells have been shown to integrate into all embryonic germ layers, including the germ line, following injection into blastocyst and develop into chimaeric animals. Some mES lines can form entire viable foetuses and newborns when injected into heat-treated blastocysts or tetraploids embryos (Amit and Itskovitz-Eldor 2002). The ability of hESCs to contribute to form chimaeric embryos for ethical reasons cannot be tested.

The derivation of the first hESC line for the first time was reported by Thomson (Thomson, Itskovitz-Eldor et al. 1998). A number of hESC lines were derived since then and at present European hESCs registry lists over 700 hESC lines. However, not all lines have been fully characterized. The defining features of ESCs include: derivation from preimplantation embryos, pluripotency, capacity for prolonged proliferation, and self-renewal, a normal euploid karyotype and the expression of distinctive

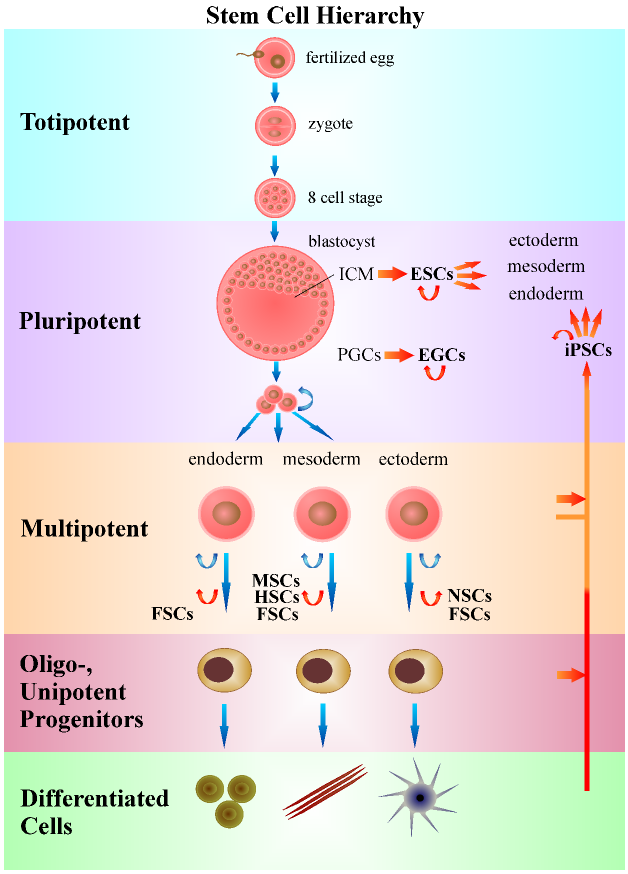

Figure 1. Stem cell hierarchy

markers. There are several methods of hESCs isolation, each having certain advantages and disadvantages. In general, the most common method in majority of laboratories is the immunosurgical isolation of ICM. It is based on the lysis of trophectoderm using the antibody/complement reaction. In this process the trophoblast layer of the blastocyst is selectively removed and the intact ICM is further cultured in mitotically inactivated mouse embryonic fibroblasts (mEFs). If the blastocyst possesses a large and distinct ICM, this method allows easy isolation of the ICM and selective removal of the trophectoderm from expanded blastocysts; although, in blastocysts with small or indistinct ICM there might be difficulties with ICM isolation. Another drawback of this method is a possibility of contamination with animal pathogens. To circumvent these problems the partial and whole embryo culture methods can be used. In the whole embryo culture method the entire blastocyst without zona pellucida is seeded directly onto the feeder layer. This method can be used regardless of the blastocyst quality; however, there is a risk of trophectodermal overgrowth, which impedes the growth of ICM. The partial embryo culture method can be used in blastocysts with small ICMs. In this method the region containing ICM is removed with an ultrafine glass pipette, therefore eliminating the overgrowth of the trophectoderm (Moon, Park et al. 2006).

ECSs grow as tightly compacted colonies of undifferentiated cells. To maintain their undifferentiated state, ESCs are typically grown on the feeder cell layers of mEFs. The feeder layer has a dual role: firstly, it supports ES cell growth; secondly, it prevents ESCs from spontaneous differentiation. Mouse hESC cultures are also supplemented by the antidifferentiation cytokine leukemia inhibitory factor (LIF). Human cultures do not have the same response to LIF, instead they are maintained in the media containing foetal bovine serum or serum replacement media complemented with basic fibroblast growth factor. When ESCs are removed from these conditions, they undergo spontaneous differentiation.

During hESCs culture on MEFs with medium supplemented with FBS there is a risk of exposing the cells to retroviruses or other pathogens. Since their first derivation in 1998, a number of improvements to the basic culture conditions were proposed, therefore enabling the isolation and culture of hESC suitable for clinical applications. The first step towards this solution was made by Xu (Xu, Inokuma et al. 2001), who proposed a culture system in which hESCs were grown on matrigel, laminin, or fibronectin using 100% MEF-conditioned medium, supplemented with serum replacement. Richards and colleagues proposed to replace FBS with 20% human serum and to replace MEFs with human feeder layer (Richards, Fong et al. 2002). The combination of serum replacement, human fibronectin, and human recombinant growth factors provides safe, animal-free conditions for culturing cells, which can be used in clinic.

When the essential factors for the maintenance of undifferentiated states are removed, the ESCs start to differentiate spontaneously and to form aggregates called embryoid bodies (EB). At present, there are several ESCs differentiation approaches: the first is through induction of EB formation; the second is via co-culturing with specific feeder cells; and the third method is via differentiating ESCs in a monolayer on defined structures. The last protocol is the simplest and it also minimizes influences of unknown factors, influencing differentiation (Moon, Park et al. 2006).

The existing hESC lines were derived in different laboratories using different techniques for derivation and subsequent cell culture which may result in SC lines with different qualities. Increasing interest and constant rise of stem cell lines lead to the need of creating of stem cell banks. Cell banks, storing and distributing human embryonic stem cells have been established in a number of countries (Holm 2016). A number studies attempted to characterize and compare different hESC lines; however the most comprehensive assessment of the hESCs phenotype (including analysis of 59 hESC lines) was performed by International Stem Cell Initiative (ISCI). ISCI is a worldwide collaborative effort aiming to establish consensus on basic criteria and techniques for hES and hiPS cells and their applications in human medicine. The work has been conducted in several stages and covered the topics of antigen and gene expression patterns of hES cells, comparison of culture media and genetic changes that occur during prolonged growth of hES and hiPS cells and finally development of consensus protocols for assessing pluripotency. To date hESC lines (like other SC lines) have been characterized by their developmental potential, transcriptional and epigenetic

profiles and cell-surface markers. The criteria of this assessment include the expression of surface markers and transcription factors associated with undifferentiated state. In addition the proliferative capacity, pluripotency and euploid karyotype and epigenetic status are being assessed (Hoffman and Carpenter 2005).

Several approaches have been used to characterize hES cells, but the most widespread are analyses of cell surface–antigen phenotype, often by flow cytofluorimetry, and gene expression studies, commonly assessed by reverse transcription-polymerase chain reaction (RT-PCR) and or by microarray analyses. These methods are first, and very often the only, methods applied to characterize stem cells in general, both undifferentiated and during differentiation.

Stem cell markers

Identification of reliable markers for the characterization of hESCs is of great importance in order to exploit their potential. The fact that mouse and human ESCs do not have the same response to LIF indicates that they may require different signals to maintain their pluripotency and self-renewal. Indeed, the comparison of expression patterns of mouse ESCs and hESCs demonstrated the existence of both similarities and differences between them (Sato, Sanjuan et al. 2003). For example they both express alkaline phosphatase-related antigens. Although mESCs express SSEA-1, while hESCs express SSEA-3 and SSEA-4, but not SSEA-1. In general the SSEA-1(-)/SSEA-3(+)/SSEA-4(+) phenotype of hESCs versus SSEA-1(+)/SSEA-3(-)/SSEA-4(-) phenotype of mESCs was confirmed (Adewumi, Aflatoonian et al. 2007). A number of surface markers are currently used to characterize hESCs. They include glycolipid antigens SSEA-3 and SSEA-4, keratin sulphate antigens TRA-1-60 and TRA-1-81, GCTM2 and GST343, the protein antigens CD9, Thy1 (also known as CD90), tissue-nonspecific alkaline phosphatise and class 1 HLA (Adewumi, Aflatoonian et al. 2007).

Gene expression

The gene expression profiling is being performed in order to characterize the undifferentiated pluripotent state of ESCs. Although the differences between hESC lines have emerged, there are numerous commonalities. The ISCI determined similar expression patterns for several genes in all 59 hESC lines tested. These strongly developmentally regulated genes were NANOG, POUF5F1 (formerly known as OCT4), TDGF1, DNMT3B, GABRB3 and GDF3 (Adewumi, Aflatoonian et al. 2007). NANOG, POUF5F1 and TDGF1 are genes associated with the pluripotent state. The POUF5F1 transcription factor (Oct-4), is expressed in pluripotent cell populations such as ES, EC, and embryonic germ cells and is downregulated upon DNMT3B gene encodes DNA methyltransferase which is thought to function in CpG methylation, an epigenetic modification that is important for embryonic development, imprinting, and X-chromosome inactivation. GDF3 belongs to the TGF-β superfamily and is thought to potentiate NODAL.

Pluripotency analysis

Pluripotency is one of defining features of ESCs. The most definitive test to validate pluripotency is the formation of chimaeras in mice. For this purpose ESCs are injected into the blastocyst and the contribution of the ESCs to the resulting chimaera is assessed to determine the differentiation capacity of the injected cells. As it was already mentioned, due to ethical reasons this method is not suitable for hESCs.

Two tests are being used currently to validate the pluripotency of hESCs. These are: embryoid body formation in vitro (italics) and teratoma formation after injection into immunocompromised mice. Both will differentiate and contain cells from all three germ layers, demonstrating pluripotency. EBs will develop spontaneously in ESC or iPSC cultures upon withdrawal of factors that maintain pluripotency, when cultures become very dense, or when suspended in the media that promotes formation of EBs. Teratomas are tumors containing differentiated and undifferentiated cells from all the three germ layers. Generation of teratomas is a method for functional analysis of pluripotent stem cells italics. Alternatively, generation of specific functional cell types from hESCs can also be demonstrated both italics.

Cytogenetic analysis

The ability of hESCs to maintain a normal karyotype throughout extended culture periods is one of the core features of ESCs. Some studies have reported abnormal karyotypes in hESCs that are similar to those abnormalities observed in EC cells, such as trisomy 12 and 17, while the expression of standard hESCs markers remain unchanged. It was also proposed that these abnormalities may be related to a manner in which the cells are passaged (For review see (Hoffman and Carpenter 2005). Thus, it is crucial to monitor a culture for the any chromosomal abnormalities, particularly in stem cells intended for therapeutic use. It is generally recommended that a stem cell line be karyotyped every 10–15 passages to ensure that chromosomal duplications, insertions, deletions, translocations, or centromere loss have not occurred. Traditional karyotyping uses dye to stain the chromosomes of a metaphase cell in distinct banding patterns. The most common method is Giemsa staining, known as G-band karyotyping or G-banding; other methods include R-banding (reverse Giemsa staining), C-banding (constitutive heterochromatin staining), Q-banding (quinacrine staining), and T-banding (telomeric staining). Changes in banding patterns are used to identify abnormalities.

Epigenetic analysis

Epigenetics is defined as the sum of processes that cause heritable and reversible changes of gene expression patterns that do not involve changes of primary DNA sequences. Stem cell renewal and differentiation requires selective activation or silencing of specific transcription

programs. Epigenetic modifications establish the memory of active and silent gene states and contribute to determination of stem cell fates (Zhou, Kim et al. 2011). Multiple lines of evidence suggest that both maintenance of stemness and lineage commitment, are tightly controlled by epigenetic mechanisms such as DNA methylation, histone modifications, changes in chromatin structure due to chromatin remodelling and non-coding RNAs, transcription factors, and miRNA. The balance between these mechanisms may determine whether the cells will differentiate or stay pluripotent as well as their direction of differentiation (Bibikova, Chudin et al. 2006). Numerous hESC lines are being derived under different conditions, perhaps contributing to potential differences in the regulation of early developmental events. Such developmental events include epigenetic phenomena such as autosomal imprinting and X-chromosome inactivation. A loss of imprinting of H9 gene after prolonged culture of H9 hESCs was shown to be critical for proper cell growth and development (Hoffman and Carpenter 2005). Therefore examination of epigenetic status of SCs is important to characterize cell lines following extended passage and/or upon cellular differentiation. Studies are now being conducted to examine the epigenetic status of hES cell lines as a means to further characterize the cells and to demonstrate that hESCs derived and maintained under varying conditions may indeed exhibit not only different characteristics, but may also respond differently to environmental stimuli. Epigenetic modifications can also influence the safety and efficacy of hESCs when used clinically. Although, the field of epigenetic regulation in SCs is very complex and for today is not well understood, it may provide the knowledge that will lead to a deep understanding of SC biology and to new simpler methods of reprogramming cells.

Induced pluripotent stem cells

iPS cells possess several advantageous features when compared with ESCs or adult MSCs. For example similar to ESCs they are pluripotent cells able to generate cells of all the three germ layers, but many ethical issues connected with hESCs can be omitted when using iPSCs instead of ESCs. The advantage of MSCs is that they are patient specific and are not tumorogenic; however, their differentiation capacity is limited. iPSCs are also patient specific and can be obtained virtually from any cell in the adult organism. Patient-specific iPSCs can also be used for drug screening purposes or for developing disease models (Takahashi and Yamanaka 2013; Freedman 2015; Xu, Huang et al. 2016). In 2006, Takahashi and Yamanaka generated induced pluripotent stem (iPS) cells by over-expressing a few types of transcription factors Oct3/4, Sox2, Klf4, and c-Myc in mouse embryonic fibroblasts (Takahashi and Yamanaka 2006). However, some key pluripotent genes were not fully activated, and, therefore in 2007 Takahashi et al and Yu et al independently from each other proposed other genes, candidates for reprogramming. The first group used Oct3/4, Sox2, Klf4, and c-Myc on human dermal fibroblasts (Takahashi, Tanabe et al. 2007) and other group used Oct3/4, Sox2, Nanog, and Lin28 on human somatic cells (Yu, Vodyanik et al. 2007).

Mesenchymal stromal cells (MSCs)

MSCs could be easily isolated from bone marrow and adipose tissue (BMSCs and AMSCs, respectively). Properties of bone marrow-derived MSCs (BMSCs) are among the best characterized and they are among the most widely used type of cells in clinical practice. Some scientists believe that AMSCs are more suitable cells for allogenic transplants and tissue engineering as they retain a stem cell phenotype and mesenchymal pluripotency through higher passages (over 25 passages) and are easier to work with (Zhu, Liu et al. 2008). Extensive growth in culture makes it possible to obtain the required number of cells for transplantation and also to graft autologous cells, thus avoiding the use of cytostatic. All MSCs can differentiate in vitro into chondrocytes, osteocytes, muscle cells, adipocytes, and even neurons and glia (Prockop 1997; Mezey, Chandross et al. 2000; Krause 2002). The plasticity (ability of the cell to change its default fate) and tissue regenerative potential of MSCs may far exceed their use in hematopoietic diseases. Grafting of MSCs induces paracrine effect (e.i. secrete growth factors such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), neural growth factor (NGF), glia cell-line derived neurotrophic factor (GDNF) and IGF-1), that play a crucial role in neuroregeneration (Gu, Zhang et al.; Uccelli, Benvenuto et al.; Li, Chen et al. 2002; Zhang, Li et al. 2004; Vercelli, Mereuta et al. 2008). Transplanted BMSC are immunopotent, do not stimulate alloreactivity, could pass through MHC barriers and be transplanted between human leukocyte Ag (HLA)-mismatched individuals (Le Blanc 2003; Aggarwal and Pittenger 2005; Urdzikova, Jendelova et al. 2006; Rice and Scolding 2008). MSCs present a very attractive source of cells for neuroprotective and reparative therapy especially considering expression of a large variety of neuronal genes and transcription factors with potential neural involvement, suggesting a wide differentiation potential (Blondheim, Levy et al. 2006; Zhu, Liu et al. 2008; Arboleda, Forostyak et al. 2011). Moreover, some in vitro experiments showed the possibility of MSCs differentiation along the neuronal pathway toward a functional phenotype (Tropel, Platet et al. 2006).

The above characteristics of MSCs could explain their positive effect on motor activity and survival after the intravenous transplantation of human umbilical cord blood (hUCB) and rodent MSC in different animal models (Mazzini, Fagioli et al. 2004; Garbuzova-Davis, Sanberg et al. 2008; Vercelli, Mereuta et al. 2008; Kim, Kim et al. 2010). The combined (intraspinal and intravenous) transplantation of rat BMSCs resulted in a neuroprotective effect, along with decreased inflammation, the attenuated proliferation of microglial cells, the reduced expression of COX-2 and NOX-2 that increases motor activity and extends the lifespan of amyotrophic lateral sclerosis (ALS) rats (Boucherie, Schafer et al. 2009; Forostyak, Jendelova

et al. 2011). Intrathecal injections of MSCs were able to preserve f and balance ECM compounds and even modify host gene expression (Forostyak, Homola et al. 2014). The effects of MSCs are dose-and passage-dependent. MSCs from earlier passages are more suitable for stem cell therapy due to their stability, anti-inflammatory and neuroprotective effects (Choi, Kim et al. 2010). A similar neuroprotective effect was achieved by the administration of one million (10 to the power of 6) cells in asymptomatic SOD1 animals, while 100 000.0 (10 to the power of 5) cells failed to extend the lifespan or to increase the motor neuron (MN) count in the same animal model (Habisch, Janowski et al. 2007; Kim, Kim et al. 2010).

Stem cell therapy

Every year an increasing number of people are affected by neurological diseases such as neurodegenerative diseases (e.g. ALS, Alzheimer disease (AD), Parkinson disease (PD), traumatic spinal cord (SCI) and brain injury; stroke and central nervous system (CNS) tumors. In this list, spinal cord injuries and ALS are among the most devastating disorders, considering patients' young age and that these people together with their families are often deprived of qualities that change their lives forever (Forostyak, Jendelova et al. 2013). Adult CNS regeneration is limited due to poor compensatory processes (spontaneous regeneration of affected axons, dendritic remodeling, changes in neuronal and synaptic strength etc.) that are taking place inside the CNS after the trauma or during neurodegeneration in order to overcome a number of neurites growth-inhibitory molecules and to restore lost structures and functions (Carulli, Pizzorusso et al. 2010). Since current therapeutic approach cannot target the above obstacles the novel therapeutic strategies should confront them, by stimulation of neural plasticity (including modification of glial scar components), providing neuroprotective support for the remaining host cells, acting as an anti-inflammatory and demyelinating agents and stimulate the intrinsic regenerative potencial of the adult CNS. Stem cells are powerful tools that possess a much bigger potential than aforementioned and, thus, are perfect candidates to be used in the therapy of CNS disorders.

General approach to stem cell therapy in the treatment of neurological diseases

Cell-based therapies have a broad field of application. Here we showed a potential of stem cell application as an example of ALS and SCI by making an overview of the results from bench-to-bedside. Generally speaking, different types of stem cells showed the ability to facilitate either restoration/substitution or the preservation of upper and lower motoneurons (MN) after the different roots of transplantation. In the first case, new/donor neurons must be integrated into existing neural circuits after transplantation (Lindvall and Kokaia 2006). Past in vitro and in vivo studies have generated MN from animals and human ESC that maintain a typical motoneuronal phenotype and show functional incorporation after intraspinal transplantation into rodents with motor deficiencies (Wichterle, Lieberam et al. 2002; Papadeas and Maragakis 2009). Various cell types, such as human neural stem/progenitor cells (hNSC) and glial restricted precursors (GRP), have been shown to ameliorate ALS, reduce MN degeneration, extend survival and even structurally integrate into the segmental motor circuitry via the formation of functional synapses with the host neurons (Xu, Yan et al. 2006; Lepore, Rauck et al. 2008; Xu, Ryugo et al. 2009). Some studies also demonstrated formation of functional connections between grafted ES cells and the host muscles after transplantation into an acute injury of the peripheral nerves of (Deshpande, Kim et al. 2006; Yohn, Miles et al. 2008). Thus, the generation and grafting of support cells aimed at protecting the remaining host MN might be more realistic and effective. It is necessary to keep in mind that the transplantation of human embryonic stem cell-derived neural progenitors might cause hyper proliferation and the formation of teratomas (Seminatore, Polentes et al. 2010). Currently, the field of stem cells research has made a huge progress toward safe application into humans but it is still ambiguous for routine clinical application in patients. The main concerns are: a risk of tumorigenesis, limited access to human material, logistical, immunological and ethical issues (Widner, Brundin et al. 1988; Vaquero and Zurita 2011; Pen and Jensen 2016).

Clinical trials of stem cell therapy of CNS disorders

The successful application of MSCs in preclinical trials established a platform for clinical studies in human patients (Vercelli, Mereuta et al. 2008). The first long term outcome after nearly 9 years of monitoring of 19 ALS patients, enrolled in two phase I clinical trials, showed no clear clinical benefits in these patients. However, the collected data show support for the implantation of autologous bone marrow MSCs into the dorsal spinal cord, as no structural changes (including tumor formation) or deterioration in psychosocial status were found, and all patients coped well with the procedure (Mazzini, Fagioli et al. 2003; Mazzini, Ferrero et al. 2010; Mazzini, Mareschi et al. 2011). Another clinical study used the transplantation of mononuclear CD133(+) autologous stem cells from the peripheral blood into the frontal motor cortex of ALS patients (Martinez, Gonzalez-Garza et al. 2009). This method of cell application significantly prolonged the survival of the treated patients and the maintenance of their lifestyle compared with untreated control patients. Deda et al. reported the results of a one year follow-up after the implantation of bone marrow-derived hematopoietic progenitor stem cells into the anterior part of the spinal cord of thirteen patients with a bulbar form of SALS: nine patients became much better compared with their pre-operative status; one patient was stable without any decline or improvement in his status; and three patients died 1.5, 2 and 9 months, respectively, after stem cell therapy as a result of lung

infection and myocardial infarction (Deda, Inci et al. 2009). A phase I/IIa clinical trial involving ALS patients and transplantation of autologous MSCs (intramuscular, intrathecal or combination of both) resulted in at least 25% improvement of ALSFRS-R score 6 months after application of cells in the slope of progression in the responders (Petrou, Gothelf et al. 2016). Some groups even reported improvement of neurological functions in stroke patients after the repeated delivery of both adult (MSCs) and fetal (NSPCs) stem cells (Qiao, Huang et al. 2014). Intrastriatal grafts of human fetal ventral mesencephalic tissue, rich in dopaminergic neuroblasts, as restorative treatment for their Parkinson disease into PD patients indicate that dopaminergic cell transplantation can offer very long-term symptomatic relief and provide proof-of-concept support for future clinical trials using fetal or stem cell therapies (Kriks, Shim et al. 2011; Kefalopoulou, Politis et al. 2014; Lindvall 2016).

Conclusions

All of the above properties of stem cells, preclinical trials, along with long experience with the transplantation using animal models of CNS diseases, lead to clinical trials, initially to treat myocardial infarction and later to treat stroke, SCI, ALS, PD and other diseases of the CNS (Bang, Lee et al. 2005; Schachinger, Erbs et al. 2006; Sykova, Homola et al. 2006). Experimental studies suggest that the therapeutic effect of grafted cells starts before the establishment of a connections suitable for the passage of axons, therefore the recovery of neurological functions at the early post-transplantation stage could be explained by the activation of different regenerative processes, mainly the release of neurotrophic factors (Zurita and Vaquero 2004). Transplantation of embryonic and iPS cells in small series of experiments involving patients showed an improvement of motor and sensory functions, significant hurdles remain before these findings can be responsibly translated to novel therapies. We still need a better understanding of the mechanisms of action and the behaviour of stem cells in the pathological environment after transplantation and clinical trials with larger and homogenous groups of patients are needed, to enable better comparison with control treatments (Lindvall and Kokaia).

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