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Embryonic Stem Cell Therapy – From Bench to Bed

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1. Introduction

The term stem cell includes a large class of cells defined by their ability to give rise to various mature progeny while maintaining the capacity to self-renew. Embryonic stem cells (ESCs) were first isolated from the inner mass of late blastocysts in mice by Sir Martin J. Evans and Matthew Kaufman (Evans & Kaufman, 1981) and independently by Gail R. Martin (Martin, 1981). Later, it became possible to obtain ESCs from non-human primates and humans. In 1998, James Thomson and his team reported the first successful derivation of human ESC lines (Thomson *et al.*, 1998), thus extending the great potential of ESCs by providing the opportunity to develop stem cell-based therapies for human disease.

Embryonic stem cells are pluripotent, a term that defines the ability of a cell to differentiate into cells of all three germ layers. There are different types of mammalian pluripotent stem cells: embryonic stem cells derived from pre-implantation embryos (Evans & Kaufman, 1981), embryonic carcinoma (EC) cells, the stem cells of testicular tumors (Stevens, 1966; Evans, 1972), epiblast stem cells (EpiSCs) derived from the late epiblast layer of post-implantation embryos (Brons *et al.*, 2007), and embryonic germ (EG) cells derived from primordial germ cells (PGCs) of the post-implantation embryo (Matsui *et al.*, 1992; Stewart *et al.*, 1994).

Besides isolating pluripotent cells from different embryonic tissues, various experimental methods are available nowadays for inducing pluripotency *in vitro*. These methods include cloning by somatic cell nuclear transfer (SCNT), cellular fusion with embryonic stem cells, the induction of parthenogenesis, and direct reprogramming by addition of reprogramming transcription factors. SCNT is done by replacing the oocyte genome at metaphase II of meiosis with a somatic cell nucleus. Although somatic cell reprogramming has been achieved in several mammalian species (Wilmut *et al.*, 1997), this seems to be very difficult to achieve in humans. Only in 2011 Noggle *et al.* (Noggle *et al.*, 2011) succeeded to generate human pluripotent cells by using SCNT. However, their study revealed that the classical SCNT consists

tently leads to developmental arrest. The activated human oocytes develop to the blastocyst stage only when the somatic cell genome is merely added and the oocyte genome is not removed. Human stem cells derived from these blastocysts contain both a haploid genome derived from the oocyte and a diploid somatic cell genome reprogrammed to a pluripotent state (Noggle *et al.*, 2011). However, the SCNT raises some ethical concerns regarding the use of human eggs. It has also been reported that somatic cells could be reprogrammed by fusion with ES cells (Do *et al.*, 2006). These cells offer a good alternative to SCNT, especially for studying the mechanisms of reprogramming, but are thought to be less interesting for therapies due to the presence of the nuclei of stem cells in the hybrids and their instability. Human ESC lines derived from parthenogenetic blastocysts obtained by artificial activation of an oocyte have been obtained (Turovets *et al.*, 2011). Their immune-matching advantage, combined with the advantage of derivation from nonviable human embryos makes these cells a good source for cell-based transplantation therapy. However, one of the most exciting reports in reprogramming was the generation of iPSCs from terminally differentiated somatic cells by transduction of four transcription factors (*OCT4*, *SOX2*, *KLF4* and *c-MYC*) into fibroblasts (Takahashi & Yamanaka, 2006).

By using various biological reagents (e.g. growth factors) (Schuldiner *et al.*, 2000), ESCs can be differentiated in the laboratory into a range of different cell types, including neurons, glia, cardiomyocytes, islet beta cells, hepatocytes, hematopoietic progenitors and retinal pigment epithelium. The ESC ability to give rise to many different cell types is the reason that makes them very good candidates for cellular therapies. Many of the diseases that place the greatest burden on society are, at their root, diseases of cellular deficiency. Diabetes, stroke, heart diseases, hematological and neurodegenerative disorders, blindness, spinal cord injury, osteoarthritis, and kidney failure all result from the absence of one or more populations of cells that the body is unable to replace. Three basic methods have been developed to promote differentiation of ESCs: (1) the formation of three-dimensional aggregates known as embryoid bodies (EBs) (Itskovitz-Eldor *et al.*, 2000), (2) the culture of ESCs as monolayers on extracellular matrix proteins, and (3) the culture of ESCs directly on supportive stromal layers (Kawasaki *et al.*, 2000; Murry & Keller, 2008). However, the controlled differentiation of ESCs is rather difficult to optimize due to the use of serum in the culture media and difficulty to select differentiated cells. In this chapter I will focus on the differentiation of ESCs into the ectodermal lineage and on the two in 2012 ongoing clinical trials involving transplantation of ESCs derivatives into eye and spinal cord.

2. Treatment of eye diseases

Retinal degenerative diseases that target photoreceptors or the adjacent retinal pigment epithelium (RPE) affect millions of people worldwide. Age-related macular degeneration (AMD) is a late-onset, complex disorder of the eye with a multi-factorial etiology in elderly (Katta *et al.*, 2009). Being the third leading cause of blindness worldwide, it accounts for 8.7% of blind persons globally. AMD results in progressive and irreversible loss of central vision affecting the macula of the eye and involves the RPE, Bruch's membrane (BM) and

choriocapillaries (Katta *et al.*, 2009). Other retinal diseases with limited conventional treatments include Stargardt's macular dystrophy (SMD) and retinitis pigmentosa (RP). SMD is the most common early-onset macular degeneration disease, usually manifesting in people between ages 10 to 20. Initially there is an abnormal deposit of lipofuscin (yellow–brown granules of pigment that manifest with age) in the RPE. The RPE eventually degrades, which leads to photoreceptor loss, causing a decrease in central vision (Rowland *et al.*, 2012). In attempts to develop cell-based therapies for blinding diseases, two different approaches have to be distinguished. The first is a more direct approach of implanting appropriate retinal or RPE precursor cells, with the hope that they may integrate autonomously into the remaining (and diseased) target tissue. The second strategy counts on a lesser degree of cell autonomy within the diseased environment. Therefore, in this case, the bioengineer will first reconstruct a piece of retina or RPE tissue *in vitro*, which then can be implanted into the lesioned or diseased location (Layer *et al.*, 2010). This approach is called tissue engineering.

Restoration of vision has focused up to now on transplantation of neural progenitor cells (NPCs) and retinal pigmented epithelium (RPE) to the retina. The retinal pigment epithelium (RPE) is a monolayer of pigmented cells forming a part of the blood/retina barrier and plays crucial roles in the maintenance and function of the retina and its photoreceptors (Strauss, 2005). The apical membrane of the RPE is associated with the rod and cone photoreceptors of the retina. The basal side of the RPE faces Bruch's membrane, thereby separating the NR from the blood. The RPE absorbs light energy to increase visual sensitivity and protect against photooxidation, transports nutrients and ions between the photoreceptors at its apical surface and the choriocapillaries at its basal surface, phagocytoses photoreceptor outer segments, according to a daily circadian cycle, to relieve the photoreceptors of light-induced free radicals. The RPE secretes a variety of growth factors, such as the neuroprotective-antiangiogenic pigment epithelium-derived factor (PEDF) which is released to the neural retina, and the vasoprotective-angiogenic vascular endothelial growth factor (VEGF) that is secreted to the choroid (Layer *et al.*, 2010). With these diverse functions of the RPE it is not surprising that dysfunction and loss of RPE leads to degeneration of photoreceptors several diseases such as age-related macular degeneration (AMD), retinitis pigmentosa and Stargardt's disease.

2.1. Preclinical work

Cell transplantation is a novel therapeutic strategy to restore visual responses. Human embryonic stem cells (hESCs) may serve as an unlimited source of RPE cells and photoreceptors for transplantation in different blinding conditions.

hESC studies have focused on the derivation of subsets of retinal cell populations (Meyer *et al.*, 2009), with emphasis on the production of either retinal progenitors (Banin *et al.*, 2006; Lamba *et al.*, 2006), or more mature cells such as retinal pigment epithelium (RPE) (Klimanskaya *et al.*, 2004) or photoreceptors (Osakada *et al.*, 2008).

Several groups have demonstrated that differentiating hESCs mimic the stepwise development of retinal cells *in vivo* (Meyer *et al.*, 2009). Furthermore, hESCs appear to respond to secreted morphogens in a manner predicted by studies of vertebrate neural induction and

retinogenesis. In particular, blockade of bone morphogenetic protein and canonical Wnt signaling is known to be important for neural and retinal patterning, and many retinal differentiation protocols call for antagonists of one or both of these pathways to be included in the culture medium (Gamm & Meyer, 2010). Furthermore, the differentiation toward neural and further toward RPE fate is augmented by nicotinamide and Activin A (Idelson *et al.*, 2009). Several hESC lines actually generate neuroectodermal progenitors by spontaneous differentiation, without the addition of specific factors. RPE cells for example, were being isolated from several spontaneously differentiating human ES cell lines (Klimanskaya *et al.*, 2004). In their hands (Klimanskaya *et al.*, 2004), RPE-like differentiation occurred independently of the presence of serum. RPE cells reliably appeared in cultures grown in the presence or absence of FBS without significant variations in RPE number or time of appearance. The independence of this differentiation pathway on either coculture or extracellular matrix suggests the involvement of other differentiation cues, such as potential autocrine factors produced by differentiating hES cells. The hES-derived RPE-like cells expressed the same makers as RPE cells, e.g. RPE65 protein and CRALBP (Alge *et al.*, 2003; Klimanskaya *et al.*, 2004).

So far, it has been shown that transplanted postmitotic photoreceptor precursors are able to functionally integrate into the adult mouse neural retina. However, photoreceptors are neurons and they need to form synaptic connections in order to be functional. This makes the cell therapy with photoreceptors more challenging when compared to RPE cells. Interestingly, a group from Japan (Eiraku *et al.*, 2011) could obtain formation of a fully stratified neural retina from by using a three dimensional ESCs culture system. The 3D organoids would open up new avenues for the transplantation of artificial retinal tissue sheets, rather than simple cell grafting.

2.2. Clinical trial

Until shortly, the most relevant clinical studies currently being conducted in patients with retinal degeneration were fetal retinal sheet transplants (Radtko *et al.*, 2008). This strategy has its basis on the fact that immature retinal sheet extends cell processes and forms synaptic connections with the degenerate host retina. The underlying principle is that the inner retinal neurons of the host remain intact and therefore only require synaptic connections with photoreceptors for visual function to be restored. One big problem for the application of photoreceptor cell transplantation is that an appropriate source of the precursor cells is required.

Advanced Cell Technology and Jules Stein Eye Institute at UCLA started two prospective clinical studies to establish the safety and tolerability of subretinal transplantation of human ESC-derived retinal pigment epithelium (RPE) in patients with Stargardt's macular dystrophy (clinical trial identifier-NCT01469832) and dry age-related macular degeneration (clinical trial identifier-NCT01344993) — the leading cause of blindness in the developed world (Schwartz *et al.*, 2012). The studies are in phase I/II, where only the safety and tolerability of human ESC-derived RPE cells is assessed. The team of researchers from ACT and UCLA reported their preliminary work in two patients, one with AMD, the other with Stargardt's macular dystrophy, being the first to publish data on the use of human ESC-derived cells in the clinic (Schwartz *et al.*, 2012).

One of the rationales behind using the eye for cell therapy is that the eye represents an immune-privileged site. The failure of the immune system to elicit an immune response in this and other such sites constitutes the hallmark of the immune privilege status (Hori *et al.*, 2010). The remarkably successful field of corneal transplantation in clinical practice is undoubtedly associated with corneal immune privilege. The subretinal space is protected by a blood–ocular barrier and the ocular fluids contain a potpourri of immunosuppressive and immunoregulatory factors that suppress T-cell proliferation and secretion of proinflammatory cytokines and inhibiting of both the cellular and humoral immune responses (Nieder Korn, 2002).

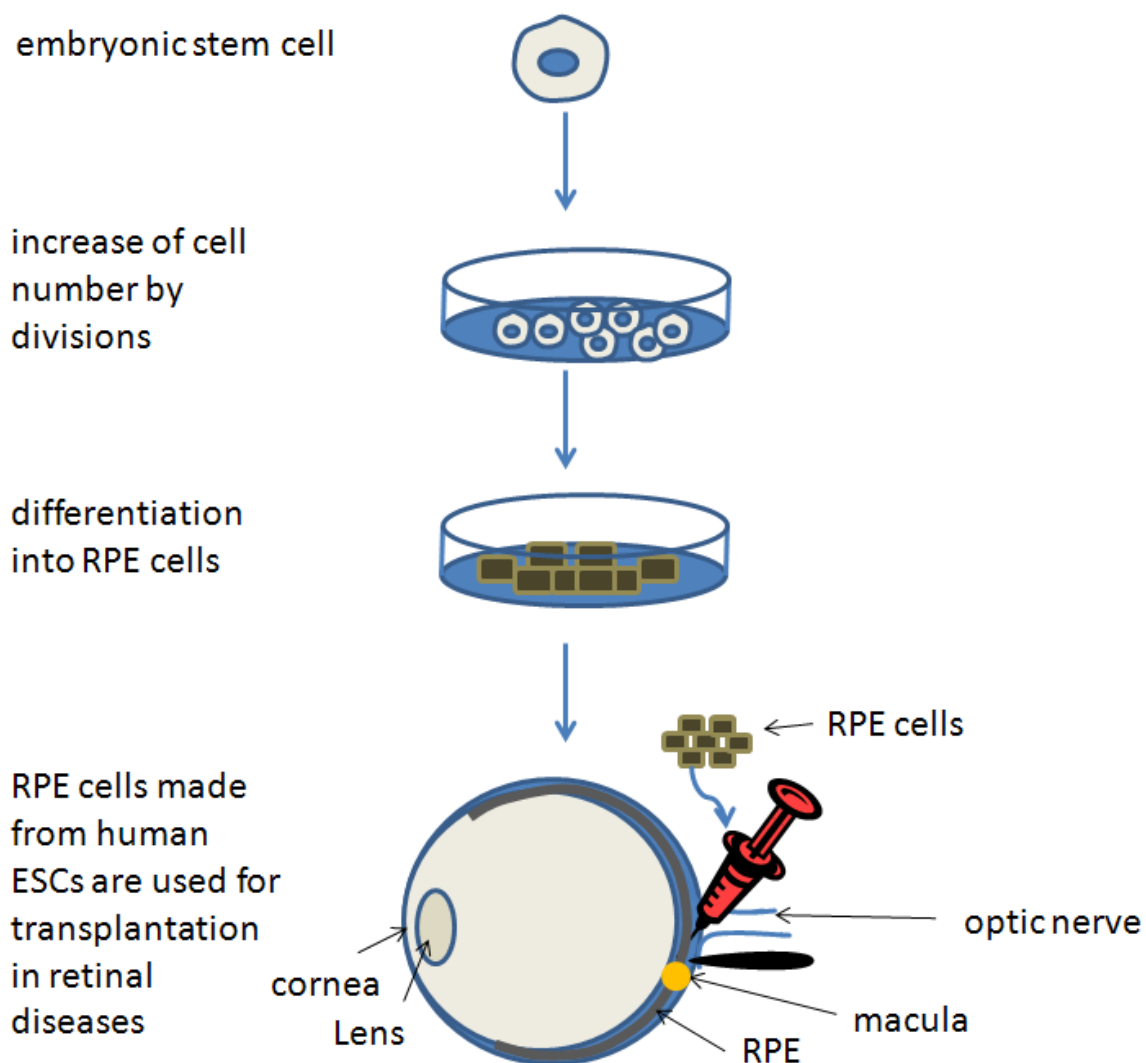


Figure 1. Scheme of procedure for replacing damaged retinal pigment epithelium cells.

Two patients enrolled in the clinical trial in order to test the safety of such cell transplantations. 50 000 viable RPE cells differentiated from the hESC line MA09 (Klimanskaya *et al.*, 2006) by embryoid body formation were injected into the subretinal space of each patient's eye (see Fig. 1 for schematic overview). The cells were resuspended in phosphate buffered saline (PBS) and delivered in a region of pericentral macula that was not completely lost to the disease. The authors thought that engraftment of the cells into a completely atrophic macula was unlikely due to the loss of Bruch's membrane. The primary outcome was positive: none of the concerns related to stem cell transplantations as teratomas, rejection, or inflammation were observed. The transplanted cells attached to Bruch's membrane and persisted for the duration of the observation period. This was however possible only in one of the two patients. Moreover, clear functional visual improvement was noted in the patient with Stargardt's macular dystrophy.

This is the first peer reviewed study that uses human ESCs for cell therapy. Although their report is preliminary, in only two patients, and with a short-term follow-up, the results are impressive - especially considering the progressive nature of both diseases (Atala, 2012).

3. Treatment of spinal cord injury

More than a decade ago, spinal-cord injury meant confinement to a wheelchair and a lifetime of medical care. Published incidence rates for traumatic spinal-cord injury in the USA range between 28 and 55 per million people, with about 10 000 new cases reported every year. Causes include motor vehicle accidents (36–48%), violence (5–29%), falls (17–21%), and recreational activities (7–16%) (McDonald & Sadowsky, 2002). The primary injury (the initial insult) is usually due to the mechanical trauma and includes traction and compression forces. Neural elements are compressed by fractured and displaced bone fragments, disc material, and ligaments and leads to injuries on both the central and peripheral nervous systems. Blood vessels are damaged, axons disrupted and cell membranes broken. Micro-haemorrhages occur within minutes in the central grey matter and spread out over the next few hours. Within minutes, the spinal cord swells to occupy the entire diameter of the spinal canal at the injury level. Secondary ischaemia results when cord swelling exceeds venous blood pressure. The more destructive phase of secondary injury is, however, more responsible for cell death and functional deficits. Hemorrhage, edema, ischaemia, release of toxic chemicals from disrupted neural membranes, and electrolyte shifts trigger a secondary injury cascade that substantially compounds initial mechanical damage by harming or killing neighbouring cells (McDonald & Sadowsky, 2002). Glutamate plays a key part in a highly disruptive process known as excitotoxicity. It was demonstrated that glutamate, released during injury, damages oligodendocytes (Domercq *et al.*, 2005). Oligodendrocytes express glutamate receptors as NMDA (Karadottir *et al.*, 2005) and AMPA/kainate receptors (Domercq *et al.*, 1999). Up to now, the primary approach in treatment is limitation of secondary injury by removal of damaging bone, disc, and ligament fragments to decompress the swollen cord, followed by the administration of the steroid methyl-prednisolone (Bracken *et al.*, 1990).

There are many repair strategies in spinal cord injury, as prevention of cell death by anti-glutamatergic drugs, promotion of axonal regeneration, compensation of the lost myelination or cell replacement therapy (McDonald *et al.*, 2002; McDonald & Sadowsky, 2002). Different sources and types of cells, including stem/progenitor cells (embryonic stem cells, neural progenitor cells, bone marrow mesenchymal cells) and non-stem cells (olfactory ensheathing cells [OECs] and Schwann cells) have been, and/or are being tested in clinical trials for spinal cord injury (Fehlings & Vawda, 2011).

3.1. Differentiation to oligodendrocytes

As mentioned before in the case of spinal cord injury, diseases of the nervous system involve proliferation of astrocytes and loss of oligodendrocytes (OLN) and the protective myelin sheath they produce. Transplantation of oligodendrocyte precursors in different animals systems show that these precursors can myelinate axons (Groves *et al.*, 1993). Thus, derivation of oligodendrocytes from ESCs has been an important goal for cell replacement therapy. The most common protocols involve an initial differentiation step to neural progenitors (Reubinoff *et al.*, 2001), followed by expansion, further differentiation, and selection. These protocols follow the differentiation steps that take place *in vivo*. During development, oligodendrocytes differentiate from precursors, which migrate and proliferate, through immature oligodendrocytes, which send out processes seeking axons to myelinate, to mature myelinating oligodendrocytes that form myelin sheaths. The precursor cells are morphologically bipolar (when migrating) or stellate (after migration). These initially differentiate into immature cells that put out processes seeking axons to myelinate, and eventually form mature cells with parallel processes myelinating up to 30 different axons (Karadottir & Attwell, 2007).

Oligodendrocytes were first efficiently derived from mouse ESCs (Brustle *et al.*, 1999), where ESCs were aggregated to embryoid bodies and plated in a defined medium that favors the survival of ES cell-derived neural precursors, followed by the expansion of progenitors in culture medium containing FGF2 and EGF, and a switch to FGF2 and PDGF to yield bipotential glial progenitors (Brustle *et al.*, 1999). These glial progenitors were transplanted into the spinal cords of rats with a genetic deficiency in myelin production, yielding myelinated fibers in the majority of animals (Learish *et al.*, 1999). Human ESCs were first shown to differentiate into oligodendrocytes by Zhang *et al.*, 2001, who used a similar strategy involving FGF treatment followed by growth as neurospheres (Zhang *et al.*, 2001). They reported the differentiation of neural precursors into neurons, astrocytes and oligodendrocytes. However, no human oligodendrocytes were detected after transplantation of neural precursors into the brains of newborn mice, although human neurons and some astrocytes were found to have engrafted (Zhang *et al.*, 2001).

The first detailed protocol for directed differentiation of oligodendrocytes from human ESCs was published in 2005 and involved the induction of neural lineage by retinoic acid treatment, followed by expansion and selection in various media containing the differentiation factors triiodothyronine hormone, FGF2, EGF, and insulin (Nistor *et al.*, 2005). After 42 days of culture, the desired cells were found in yellow spheroids, which upon differentiation as

low-density monolayers formed 85%–95% oligodendrocytes expressing typical markers as GalC, RIP, and O4. Human embryonic stem cell (hESC)-derived oligodendrocytes were able to integrate, differentiate and display a functional myelinating phenotype following transplantation into the shiverer mutant mouse (Nistor *et al.*, 2005). Recently, other protocols were developed for generation of oligodendrocytes from ESCs. The Neman and de Vellis (Neman & de Vellis, 2012) laboratory has reported usage of defined serum-free media together with morphogens, as retinoic acid and sonic hedgehog, to devise a new method to derive a pure population of OLN from ESCs. These experiments show that human oligodendrocytes can be generated in large numbers and used to restore myelination under some circumstances in mice.

3.2. Clinical trial

In October 2010 the world's first clinical trial using human embryonic stem cells began, using ESCs converted into OLN precursor cells. The feasibility of the treatment was proofed by a wide range of pre-clinical studies that have shown that human oligodendrocyte progenitor cells implanted after spinal cord injury in rodent models show functional improvement (Keirstead, 2005; Keirstead *et al.*, 2005; Sharp *et al.*, 2010). Geron of Menlo Park, California, is the biotech company that received FDA approval to proceed with clinical trials that transplant cells derived from embryonic stem cells into the spinal cord (Alper, 2009). This company has pioneered translational research into human ESC therapies. The Geron trial (trial identification number NCT01217008), which was originally approved by the FDA, but then halted due to concerns of abnormal cyst formation, was reinitiated and approved for phase I clinical trials in the U.S. in October 2010. The trial was suspended following news that animals in a dose-escalation study developed microscopic cysts in regenerating tissue sites. In november 2011 Geron announced that it is dropping its entire program owing to financial concerns and started looking for partners for stem cell treatments and decided to not further invest in the clinical trials involving treatments with ESCs.

The trial was planned to involve treating ten patients who have suffered a complete thoracic-level spinal cord injury in a phase 1 multicenter trial. The pioneering therapy is Geron's 'GRNOPC1 product', which contains hES cell-derived oligodendrocyte progenitor cells that have demonstrated remyelinating and nerve growth-stimulating properties. In the human SCI lesion site, it is hoped that OLN precursors will work as a "combination therapy" - phenotypically replacing lost oligodendrocytes and hence remyelinating axons that have become demyelinated during injury, as well as secreting neurotrophic factors to establish a repair environment in the lesion (Hatch *et al.*, 2009). The ESCs were differentiated into OLN precursors (Hatch *et al.*, 2009) and one injection of 2 million GRNOPC1 cells was administered within 2 weeks in patients with thoracic spinal cord injury (Fig. 2). No serious adverse effects were observed in the 2 patients enrolled, only one of the patients experienced some side effects due to the immunosuppression (Watson & Yeung, 2011). However, the data generated by Geron for the FDA are not published and no preliminary report on the safety of their product is available up to now.

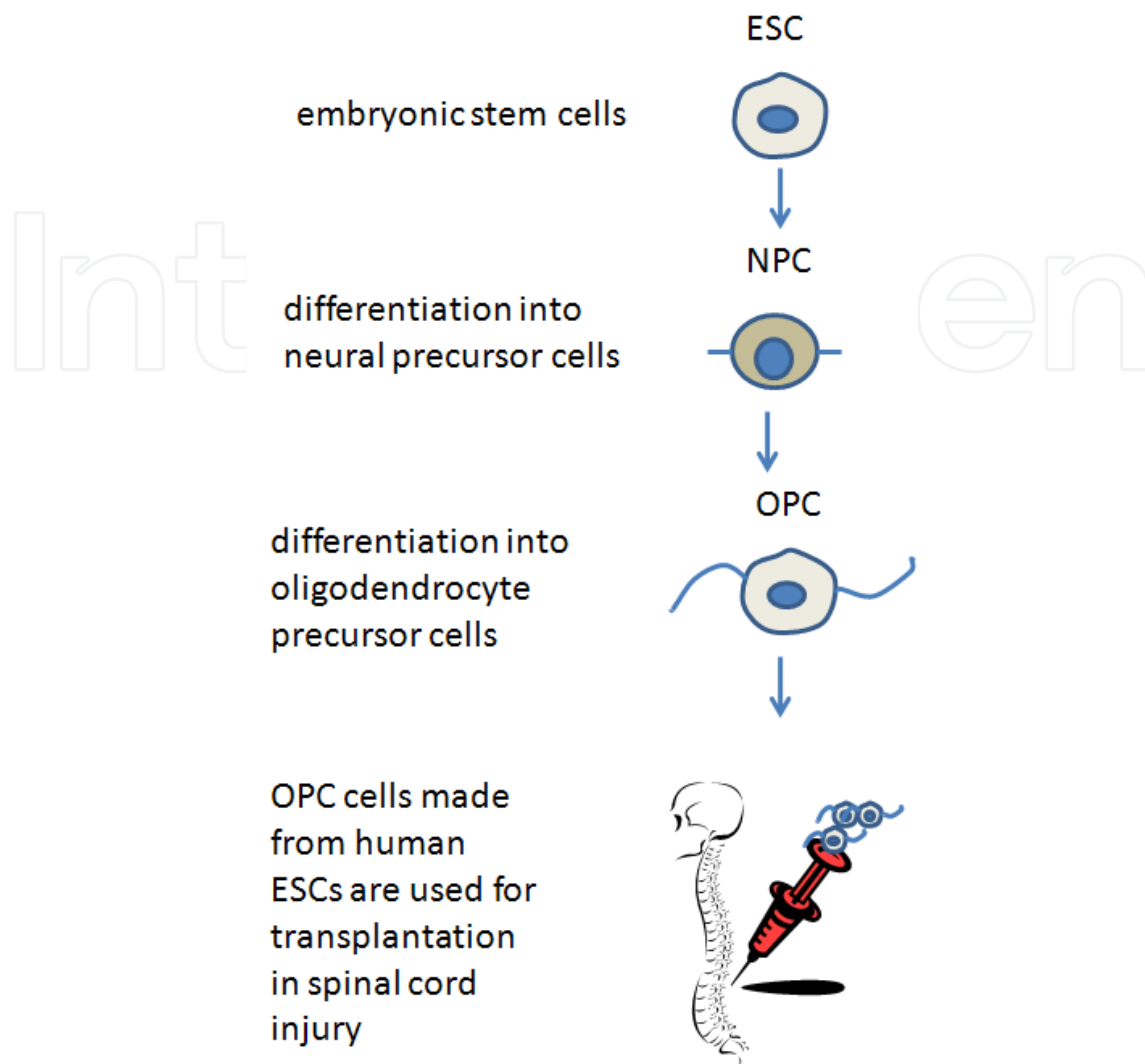


Figure 2. Scheme of procedure for treating spinal cord injury with human ESCs derived oligodendrocyte precursor cells.

4. Embryonic stem cells and tumorigenesis

The major safety concerns for the use of hESCs are related to the achievement of xenobiotic-free culture conditions, avoidance of genetic abnormalities, development of good differentiation and selection protocols, and the avoidance of the immune rejection. Moreover, the unlimited proliferative capacity of ESCs is a disadvantage in clinical applications because this could cause tumor formation upon transplantation. When implanted in an undifferentiated state, ESCs cause teratoma, a tumor type that consists of different kinds of differentiated cells. Teratomas are encapsulated, usually benign tumors that can occur naturally, but there is the fear, based on some animal studies, that some proportion of the cells derived from ESCs injected into the body could drift from their intended developmental pathway. Teratoma formation was reported in various cases when mouse

ESCs-derived cells like insulin producing islets (Fujikawa *et al.*, 2005), ESC-derived cardiomyocytes (Cao *et al.*, 2006), and ESC-derived neurons (Schuldiner *et al.*, 2001) were transplanted into immunosuppressed mice even though there was successful engraftment and functional improvement. When undifferentiated human ESCs were injected into the hind limb muscles or under the kidney capsule of SCID mice, teratomas were readily formed after 8–12 weeks (Richards *et al.*, 2002). Evidence of tumor formation has also been observed in differentiated hESC derivatives transplanted *in vivo* (Roy *et al.*, 2006). In another study, successful hESC-derived neuronal engraftment in a Parkinsonian rat model did not yield teratomas after 12 weeks (Ben-Hur *et al.*, 2004). When hESC-derived osteocytes or cardiomyocytes were transplanted into the bone or heart of severe combined immunodeficient mice (SCID), there was also no teratoma production within 1 month after injection (Bielby *et al.*, 2004; Laflamme *et al.*, 2007). It seems that the longer hESCs are differentiated *in vitro*, the risk of teratoma formation appears to be reduced. Certain sites appear to favor the growth of teratomas, while others do not, confirming a phenomenon already described that tumorigenesis of ESCs is site dependent. For example the rate of teratoma formation with hESCs in immunodeficient mice was subcutaneously 25–100%, intratesticularly 60%, intramuscularly 12.5% and under the kidney capsule 100% (Prokhorova *et al.*, 2009). Furthermore, tumor formation in the lung and thymus had the highest probability of teratoma formation while the pancreas was partially site-privileged (Shih *et al.*, 2007). Shih *et al.* observed an aggressive growth of tumors when human ESCs were injected into engrafted human fetal tissues in SCID mice (Shih *et al.*, 2007).

The simplest way to slow or even eliminate the tumorigenicity of normal stem cells prior to transplantation may be to take advantage of pluripotency by partially differentiating them into progenitors. Therefore, a promising proposed method for making stem cell-based regenerative medicine therapies safer may seem paradoxical: to not transplant stem cells at all into patients. The idea is to use the stem cells to produce progenitor or precursor cells of the desired lineage and then transplant progenitors purified by sorting (Knoepfler, 2009). This approach was presented in this chapter and is actually used in the clinical trial with oligodendrocyte progenitor cells. However, not only the embryonic stem cells, but also the implanted precursor cells seem to form teratoma in some cases. A group of Israeli researchers reported that a boy with ataxia telangiectasia who had received several fetal neural stem cell transplants developed teratomas in his brain and spinal cord four years after treatment (Amariglio *et al.*, 2009). For this reason is very important to achieve a 100% pure population of differentiated cells when using ESCs for cell therapy.

Currently, the only way to ensure that teratomas do not form is to differentiate the ESCs in advance, enrich for the desired cell type, and screen for the presence of undifferentiated cells. The elimination of undifferentiated hESCs may best be achieved by (1) destroying the remaining undifferentiated hESCs in the differentiated tissue population with specific agents or antibodies, (2) separating or removing the undifferentiated hESCs from the differentiated cell population, (3) eliminating pluripotent cells during the differentiation process, and (4) inducing further differentiation of left-over rogue undifferentiated

hESCs (Bongso *et al.*, 2008). It is also very important to develop very good and reliable methods to detect residual ESCs contamination in ESCs derived cells prior to clinical application. In their review, Fong *et al.* (Fong *et al.*, 2010) presented some available methods for the elimination of undifferentiated ESCs. These included single cell propagation with encapsulation, usage of density gradients, MACS and FACS, usage of tumor privileged sites, usage of antibodies against undifferentiated ESCs, prolonged differentiation *in vitro* before transplantation or destruction of teratoma after engraftment. However, because differentiation is not an on/off process, it is probably the best to use a combination of these methods in order to do safe cell therapy.

5. Embryonic stem cells versus induced pluripotent stem cells in clinics

Induced pluripotent cells (iPS) are generated by re-engineering mature, fully differentiated cells (e.g. human skin fibroblasts) by modifying the cells with a set of transgenes (Takahashi & Yamanaka, 2006; Takahashi *et al.*, 2007). Induced pluripotent stem cells, created by turning back the developmental clock on adult tissues, display similar gene-expression patterns to ESCs, and can produce various tissues in the human body. However, iPS cells have a major advantage over ESCs; they can be obtained directly from the individual that has to be treated. Thus, as a source of cells for therapy, they are able to avoid the immunocompatibility issues. Furthermore, the utilization of these stem cells in both clinical and basic research studies does not face ethical and political issues that otherwise surround the use of embryonic stem cells.

During the last years various studies reported the differentiation of iPS cells to various types of cells *in vitro* and these cells were used for cellular therapy in various mouse models (Wernig *et al.*, 2008; Saha & Jaenisch, 2009).

However, before bringing these cells into the clinics, their safety should be tested. For example, the initial enthusiasm related to bringing iPS cells into clinics dampened when it was shown that these cells develop teratoma more efficiently than ESCs (Gutierrez-Aranda *et al.*, 2010). It was also shown that iPS retain the epigenetic memory of the cells from which they are derived; this fact makes them to preferentially differentiate into the cell lineage from which they came from. Future clinical applications will demand new techniques for generating factor-free iPS cells such as virus-free or DNA-free approaches at acceptable efficiencies. There are also other disadvantages in using iPS cells in the clinics. Usually, they are made by integrating retroviruses into the cells as shuttle for the reprogramming factors. This problem may be solved by transient gene transfer or by delivering the pluripotency factors in protein form (Murry & Keller, 2008). The second is that iPS cells are not an “off-the-shelf” product and would likely only be produced after the patient becomes ill, precluding their use in the acute phase of the disease (Murry & Keller, 2008). Quality control is will also be difficult and expensive, because a separate batch of iPS cells would have to be made for each patient.

6. Conclusion

There is no doubt that after the hurdles are overcome, hESC-derived cells have a promising future for transplantation therapy given the versatility of these cells. It is very encouraging to see that clinical trials involving the use of hESCs have begun, and that extensive efforts are underway to efficiently, and safely differentiate hESCs into specific cell types.

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