● PERSPECTIVES

The potential of stem cell-based therapy for retinal repair

Like injured neurons in the brain or spinal cord, neurons in the retina are incapable to regenerate following injury and ultimately would lead to irreversible neuronal loss and vision impairment. Over decades, extensive effort has been made to develop strategies to protect retinal neurons from death; however, the outcome is limited (Pettmann and Henderson, 1998; Bahr, 2000; Lagali and Picketts, 2011). Replacing the degenerated retinal neurons by newly generated and functional neurons would be an ideal scenario. The rapid development of stem cell biology has recently demonstrated that stem cells could be a potential source of cells for cell replacement therapy because these cells have the self-renewal capacity and could be differentiated into many cell types. This review will discuss the therapeutic potential of stem cell-based therapy to retinal degenerative diseases.

Introduction

Retinal degeneration has been known to be caused by genetic mutation (Sullivan and Daiger, 1996; Sohocki et al., 2001; Lee and Flannery, 2007), trauma (Chang et al., 1995; Sebag and Sadun, 1996) or infection (John et al., 1987; Miller et al., 2004; Robman et al., 2005) that will lead to irreversible neuronal loss and even blindness. Other than these factors, environmental influences such as ultraviolet radiation (Taylor et al., 1992) and oxidative stress (Venza et al., 2012) could also bring forth retinal degeneration. Retinal ganglion cells and photoreceptors are the two major retinal cell types subjected to degeneration in retinal diseases. Age-related macular degeneration, cone dystrophy and retinitis pigmentosa are the common photoreceptor degenerative diseases that are the major leading cause of blindness worldwide (Hageman et al., 1995; Sohocki et al., 2001; Congdon et al., 2003; Huang et al., 2011). Glaucoma, optic neuritis and post-traumatic optic injury are the common retinal diseases leading to degeneration of retinal ganglion cells (RGCs) and their axons (Quigley et al., 1989; Quigley et al., 1995; Kerrigan-Baumrind et al., 2000). To achieve the goal of stem cell-based therapy, the survival and integration of transplanted cells are critical. To evaluate the potential of stem cell therapy for neurodegenerative disease in central nervous system, retina may be a good choice to be considered because it is an easily accessible organ. In addition, the cornea clarity makes possible for longitudinal imaging the transplanted cells and measuring the retinal function by non-invasive approaches. In contrast to the complex retinal structure, analyzing the integration and functional connection of transplanted cells to the host cells in the spinal cord could be simpler. In this regard, spinal cord may be more feasible in terms of simplicity of the cellular system.

In the clinic, non-invasive tools monitoring retinal changes and retinal activity such as optical coherence tomography and electroretinography, have been well established and commonly used. Accumulating studies showed that some success of stem cell-based therapy for replacing retinal pigment epithelium (RPE) (Idelson et al., 2009; Lu et al., 2009) or photoreceptors (Kicic et al., 2003; MacLaren and Pearson, 2007; Lamba et al., 2009; Wang et al., 2010) in animal models of retinal degeneration that prompt the design of early clinical trials (A service of the U.S. National Institutes of Health; Martell et al., 2010; Trounson et al., 2011; Schwartz et al., 2012). To replace the degenerated retinal cells, delivering cells *via* subretinal injection is a straight forward and logical approach. In this review, the potential of stem cell-based therapy using embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and retinal progenitor cells on photoreceptor degeneration diseases will be described.

Potential use of stem or progenitor cells in the treatment of retinal degenerative diseases
Embryonic stem cells (ESCs)
ESCs are pluripotent cells that are derived from the undifferentiated mass of cells in blastocyst at pre-implantation stage. The ESCs have self-renewal ability and could be differentiated into all cell derivatives from ectoderm, mesoderm and endoderm. Thus ESCs could generate any cell types that could be used for cell replacement therapy. Human embryonic stem cells (hESCs) can be obtained from 5-day-old blastocyst stage from extra *in vitro* fertilized eggs called surplus *in vitro*-producing embryos, that were originally generated for *in vitro* fertilization purpose (Thomson et al., 1998). In 1998, successful isolation and generation of hESCs line was first accomplished by James Thompson. Following that, the next question is how to differentiate these cells into specific cell type for therapeutic purpose. Significant progress has recently been made to uncover the developmental stimuli that drive pluripotent stem cells to differentiate into various neurons including retinal neurons (Jin and Takahashi, 2012) and retinal pigment epithelium (RPE) *in vitro* (Lamba et al., 2009; Amirpour et al., 2012). With these information and techniques, hESCs could be a promising source of cells for replacement therapy in patients with retinal degenerative diseases (Rowland et al., 2012).

Nevertheless, cautions should be taken that the hES cell lines and the hESCs derived cells should be fully characterized for the safety purpose. It has been reporties that individual ES cell line may has different abilities or properties of differentiation (Osafune et al., 2008). In addition, accumulating evidence showed that chromosomal errors such as aneuploidy (Hassold and Hunt, 2001; Munne et al., 2002) and mitochondrion DNA defects (Keefe et al., 1995) were found in ES cell lines. It may be because most ES cell lines were derived from surplus *in vitro*-producing embryos of infertile patients. Maintaining ES cell lines *in vitro* may affect stability. Extended culture of ES cell lines may lead to karyotype instability (Amit et al., 2000; Amit et al., 2003; Draper et al., 2004a). For example, chromosomal abnormality were revealed in three independent ES cell lines that showed gain of chromosome 17q and presence of isochromosome 12p (Draper et al., 2004b). Overall, the selection and maintaining of ES cell lines could play a very critical role to the health and differentiation property to specific cell type for therapeutic purpose.

The safety and tolerability study from the first clinical study of subretinal transplantation of hESCs-derived retinal pigment epithelium (hESCs-RPE) into patients with advanced stage Stargardt's macular dystrophy and dry age-related macular degeneration (AMD) was reported in 2012 (Schwartz et al., 2012). The hESCs line used in this trial was produced with Good Manufacturing Practice and the derived RPE cells were thoroughly examined *in vitro*. The hESCs-RPE cells were characterized to have normal karyotype, free of pathogens and contaminating ESCs or pluripotent cells. Briefly, the cells were injected into the submacular space following a vitrectomy procedure. The clinical observation showed the transplanted cells survived for at least four weeks. No sign of ocular tumor or teratoma formation and clinically significant inflammation were observed. Improved visual performance was even observed in these patients. The best-corrected visual acuity was improved from hand motions to 20/800 (and improved from 0 to 5 letters on the Early Treatment Diabetic Retinopathy Study [ETDRS] visual acuity chart) in the patient with Stargardt's macular dystrophy, and vision also seemed to improve in the patient with dry age-related macular degeneration (from 21 ETDRS letters to 28). It suggested that the hESCs derived RPE cells might be safe to patients, and may even improve their vision. Now, the multicenter Phase I/II clinical trial is ongoing and more results are eagerly awaited (A service of the U.S. National Institutes of Health).

To mimic the natural structure of RPE cell layer, hESCs-RPE cells were shown to grow into monolayer on a thin sheet of polymer (Carr et al., 2013). This approach aims to overcome the disorga-

nized fashion in which RPE cells adhere to Bruch's membrane when injected as a cell suspension. The polymer is also designed to act as a replacement for the aged and thickened Bruch's membrane usually occur in macular degeneration diseases, and thus provides an anchor for the RPE cells as well as aiding in cell delivery. It was shown in animal studies that polarized monolayers of hESCs-RPE could have better survival than cell suspensions following transplantation. No teratoma or any ectopic tissue formation was detected in the implanted rats. It suggests that animal studies may provide insights of the safety outcome before transplanting the hESCs-RPE cells to patients (Diniz et al., 2013). A clinical trial of transplanting hESCs-RPE along with polymer to AMD patients is currently on going (Carr et al., 2013; Ramsden et al., 2013).

iPSCs
Use of hESCs in animal models or patients may raise ethical and religious concern because it requires destroy human embryos. Recently, a new type of pluripotent cells was generated by reprogramming somatic cells, called iPSCs. Like hESCs, hiPSCs also have self-renewal capacity and are able to differentiate into any cell types including retinal neurons (Koch et al., 2009). Reprogramming somatic cells to iPSCs could generate patient specific iPSCs; thus, no immune rejection is anticipated. In addition, generation of iPSCs does not involve embryos.

In 2006, a milestone study by Yamanaka's group in Japan, iPSCs were first generated by introducing four stem cell factors (Oct3/4, Sox2, c-Myc and Klf4) into mouse somatic cells by retrovirus (Takahashi and Yamanaka, 2006). One year later, two independent groups of Yamanaka (Takahashi and Yamanaka, 2006; Takahashi et al., 2007) and Thomson (Yu et al., 2007) successfully generated human iPSCs by introducing similar kind of stem cell factors into human fibroblast. Generally, the rate of iPSCs production is low (< 1%). There is also a risk of tumor formation because during reprogramming, the stem cell factors will integrate into the genome of somatic cells *via* retroviral system (Selvaraj et al., 2014). In addition, comparing mouse iPSCs generated from various origins, Miura et al. (2009) showed that iPSCs derived from tail-tip fibroblasts showed residual pluripotent cells after 3 weeks of *in vitro* differentiation and later form teratoma following transplantation of the differentiated cells into immune-deficient mouse. It suggests that the properties and safety of human iPSCs from various origins should also be carefully examined.

To improve the rate and safety of iPSCs production, other alternative approaches have been recently developed using small molecule (Jung et al., 2014) and non-viral methods (Kaji et al., 2009; Lieu et al., 2013; Phang et al., 2013). In general, plasmid-induced iPSCs generation has about 1,000 fold less efficient than the viral approach (Okita and Yamanaka, 2011). Recently, it was reported that the dosage of specific reprogramming factor could affect the induction of iPSCs. Papapetrou et al. (2009) showed increased 3 fold expression of OCT3/4 in human fibroblast could enhance the iPSCs generation by 2 fold. Interestingly, excess addition of OCT3/4 would have opposite effect. On the other hand, overexpressing other reprogramming factors such as Nanog, c-Myc and Klf4 could inhibit the induction of iPSCs (Mitsui et al., 2003). It suggests that the balance on the expression of reprogramming factors is important for induction of iPSCs.

Although iPSCs appear as a promising source of cells for therapeutic use, it still needs to be further characterized with regard to some critical issues including the cellular effect of reactivation of intrinsic pluripotency and possible alterations in target cells, before moving forward for clinical use. In particular, iPSCs appear to have a greater propensity for genomic instability than ESCs and with a higher rate of point mutations (Gore et al., 2011). A global epigenetic study showed higher DNA methylation was detected in iPSCs than its origin (Deng et al., 2009; Doi et al., 2009). The abnormal methylation pattern (hypo- or hyper-methylation) may affect the differentiation property of iPSCs. Other than genomic instability and epigenetic changes, parental source of iPSCs could also affect the differentiation property. For example, iPSCs generated from peripheral blood cells could differentiate into hematopoietic lineage with high efficiency but differentiate into neurons with low efficiency (Kim et al., 2010). It suggests that iPSCs may retain some memories from their parental source. Since the process of reprogramming affects only the nuclear genome, leaving the mitochondria unaltered, the extent to which an aged or altered mitochondrial genome will influence the properties of iPSCs and their derivatives that remains to be evaluated (Koch et al., 2009).

Nevertheless, accumulating studies in animal models suggested that use of iPSCs is a feasible approach to treat neurodegenerative diseases. The first clinical trial of transplanting sheets of RPE cells derived from hiPSCs to age-related macular degeneration patient has recently been approved and will be led by Masayo Takahashi at Riken Institute (Song et al., 2013). The study is planned for 2014 (http://www.riken.jp/en/pr/press/2013/20130730_1/). It is an important step; at least, to investigate if it is safe to use iPSCs-derived RPE cells in patients.

Retinal progenitor cells (RPCs)
RPCs are stem-like cells found in immature retina including human. RPCs are comprised of an immature cell population that is responsible for the generation of all retinal cell types during development (Reh, 2006) and also retinal supporter cells such as Müller cells *in vitro* (Chow et al., 1998; Tropepe et al., 2000). Note RPCs are not a single cell type but rather a variety of cells at different stages along with incompletely characterized differentiation pathways (Mayer et al., 2005). Similar to neural stem cells, RPCs have the self-renewal ability *in vitro* but with a restricted ability of differentiation into retinal neurons (Das et al., 2005). It suggests that successful isolation and expansion of RPCs could be a potential source of cells to treat retinal degenerative diseases.

Animal studies showed that following subretinal transplantation, the RPCs could migrate and integrate into mouse (Pearson et al., 2012; Barber et al., 2013) and swine retina (Wang et al., 2014) to certain extent. The age of donor cells in mouse may play a role in the efficacy of survival and integration of transplanted cells in the host retina (Kinouchi et al., 2003; West et al., 2012). Instead of transplanting cell suspension, transplanting cells with a scaffold, may improve the survival and differentiation of transplanted cells (Tomita et al., 2005; Hynes and Lavik, 2010). Recently, packaging RPCs with scaffold or biodegradable polymer was demonstrated to promote integration (Yao et al., 2011) and differentiation of RPCs to photoreceptors *in vivo*. It suggests that transplantation of RPCs *via* an appropriate scaffold may improve the outcome of transplantation. Recently, an early clinical study of transplanting human PRCs into retinitis pigmentosa patients led by Henry Klassen, is anticipated to begin in late 2014 (www.cirm.ca.goc). We are looking forward to the outcome of the study.

Conclusions and future perspectives
Overall, the results of transplanting progenitor cells or cells derived from stem cells into retina of animal models and patients undergoing photoreceptor degeneration are encouraging. These results highlight the potential of stem cell-based therapy. Nevertheless, there are still challenges to overcome. Before evaluating any beneficial effects of stem cell-based therapy in patients, we still need substantial data from long term survival studies to show the safety of the transplanted cells. The cells derived from ESCs or iPSCs should be thoroughly characterized without contaminants such as animal derivatives and residual pluripotent cells that could potentially harm the patients. In addition, enhancing the integration and survival of transplanted cells are also critical. It may be improved by packaging cells with appropriate scaffold such as synthetic polymer, for transplantation.

Other retinal degenerative diseases targeting at retinal ganglion cells (RGCs) will be the next goal of stem cell-based therapy. Recently, iPSCs dervied retinal ganglion cells were shown to be generated (Parameswaran et al., 2010; Alshamekh et al., 2012). To achieve a

successful transplantation of stem cells-derived RGCs to patients undergoing degeneration of RGCs such as glaucoma, the stem cells-derived RGCs need to have a capacity to form precise connections to specific neurons in host retinal neurons and are also able to extend long axons along the visual pathway and ultimately, establish precise functional connection to visual targets and finally, lead to vision restoration. It is an extremely challenging task to be achieved in the future.

With regard to the rapid development of stem cell biology, it is anticipated to develop a revolutionized approach for the treatment of retinal degenerative diseases and probably, other neurodegenerative diseases in central nervous system.

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