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Research Article

An Analysis of Induced pluripotent Stem Cell (IPs) to restore the functions of human cell in the Transplantation of Glaucoma

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ABSTRACT

Introduction: Glaucoma is a heterogeneous group of optic diseases that affect almost 1% to 2% of the population older than 40 years. There are many types of glaucoma but the most common type is primary open angle glaucoma. In this study we find out the role of muller cell lines in the transplantation of glaucoma model in rats.
Material and methods: Intra ocular pressure was created with the help of laser treatment in rats. The induced pluripotent stem cells (IPs) were transplanted into the vitreous or sub-retinal space of glaucomatous or untreated eyes.
Results: The transplanted cells were surviving in-vivo for 2 to 3 weeks and reduction in graft survival was also seen at 4th week. The results explain that differentiating IPs cells within the glaucomatous eye produced cells that expressed glial cell markers.

INTRODUCTION

Glaucoma is a heterogeneous group of optic diseases that affect almost 1% to 2% of the population older than 40 years (Tielsch et al., 1991). It is estimated that 66 million people in the world suffering from glaucoma, although fewer than half of those patients do not realize that they have the disease (Quigley, 1996). There are many types of glaucoma but the most common type is primary open angle glaucoma (Rahmani et al., 1996; Quigley, 1997). One of the major risk factors for development of primary open-angle glaucoma (POAG) is elevated intraocular pressure (IOP) (Sommer et al., 1991). Vision loss caused by glaucoma is irreversible, and glaucoma is the second leading cause of blindness in the world. This disease is the main cause of blindness in many coountries.

The overall risk of developing glaucoma increases with the number and strength of risk factors. It increases substantially with the level of intraocular pressure elevation and with increasing age (Sommer et al., 1991 and Mitchell et al., 1996). Oligodendrocyte precursor cells (OPCs hold great responsibility for the generation of oligo dendrocytes in the developmental stage, whereas in adult individuals, they play a vital role in demyelinating pathologies and remyelinating of axons (Dawson et al., 2003; Watanabe et al., 2002 and Bu et al., 2004). OPCs appear to contain the majority of the stem cell characteristics (Nunes et al., 2003;Zhao et al. 2008) and has been shown to be neuroprotective in vitro (Wilkins et al., 2001).
The measurement of intraocular pressure is not an effective method for screening populations for

glaucoma. Moreover, the most commonly used method for measurement, underestimates the true intraocular pressure (IOP) of patients with thin corneas and overestimates it in patients with thick ones. Almost half of all patients with primary open angle glaucoma have pressures below 23 mmHg at a single screening (Mitchell et al., 1996). Recent findings prove the link between glucocorticoid and gluacoma. Actually, G1C1A is the first glaucoma gene which was mapped to chromosme Iq (Sunden el., 1996; Richards et al., 1994 and Sheffield et al., 1993), and mutation in this gene is responsible for autosomal dominant juvenile glaucoma (ADJG) (Stone et al., 1997; Alward et al., 1997 and Adam et al., 1997). ADJG is a rere form of glaucoma.

Stem cells have been proposed as a new approach for the regeneration of ganglion cells (Siqueira et al., 2011 and Medina et al., 2011). On the other hand, the use of other stem cells, like embryonic stem cells and neural stem cells is greatly restricted due to some ethical issues and graft rejection. Muller cells have the characteristics of stem cells and it has been reported in a wide range of mammalian species, including the adult human retina (Coles et al., 2004).

Recent studies explains that potential retinal stem cells and different types of stimuli have been shown in the muller cells of chicken and rats (Fischer et al., 2002; Lawrence et al., 2007 and Karl et al., 2008).

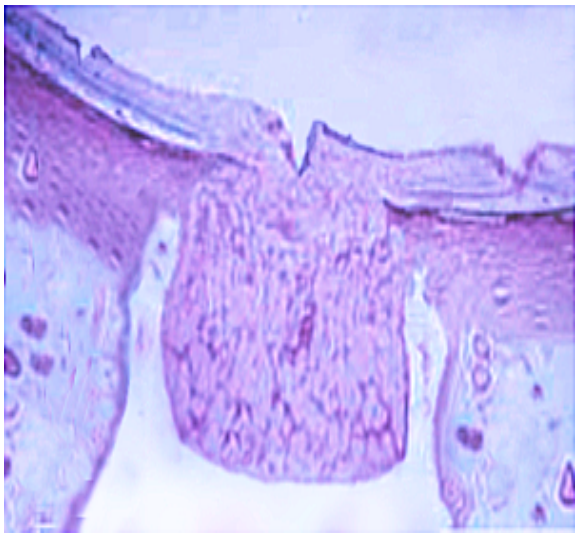

Figure 01: glaucoma in the human eye

MATERIAL AND METHODS

Ethical statement

The use of all the animals in this study was approved by the ethical committee of----------university. All the animals, albino rats (n=50) used for experiment were kept at safe place and nourished with food which is approved by FDA. All animal experiments in this study were conducted by the approval of ----------university.

EXPERIMENTAL DESIGN

First of all pilot experiment were done for the checking of graft rejection in cells but it shows that administration of cyclosporine alone were not enough for the prevention of total graft rejection. Then double therapies were used for the prevention of graft rejection. The rats were served with the mixture of two drugs in the drinking water. For this purpose cyclosporine (20mg/kg/day) and azathioprine (2mg/kg/day) were used. This drug therapy started three days before induction of glaucoma. The serum levels of cyclosporine were measured in all samples at different intervals. The average concentration was 502.2 ± 79 ug/lit.

Preparation of animals

First of all the animals were anaesthetized with the help of f ketamine and xylene. Few drops of anaesthetic agent are dropped into the eye. Then we measure the intra ocular pressure with the help of tonometer. Unilateral intra ocular pressure was induced in the eyes with the help of laser (546nm). Initial treatment consists of 60 to 65 spots of 45um diameter for 40 seconds. This laser treatment was repeated for 7 to 8 weeks after every one week. Then tissues of the eye were collected for further processing and then washed with PBS and preserve at -80ºC.

Preparation of Induced pluripotent stem cell (IPs) culture

The cell lines were prepared from postmortem human neural retina and engineered to express purple fluorescent protein to facilitate tracking on transplantation. The cell lines were purified for purple fluorescent protein for cell culturing. Cells

were used for transplantation at passages 46 to 48. The cells were maintained as an adherent cell lines in 70 cm^2 tissue culture flasks which contain D-MEM (containing 0.45 g/L glucose, sodium pyruvate and stabilized L-glutamine) and penicillin. Then cells were washed with phosphate buffer saline (PBS) then detached from the flask with the help of trypsin. Then washed the complete cell culture medium and then convert it into fresh clean flask.

HISTOCHEMICAL ANALYSIS

The cells were washed with PBS and then blocked with PBS-triton whose concentration is 0.5%. All the monoclonal antibodies were diluted with this blocked. Then this whole section was incubated at 4ºC overnight in an incubator. After incubation cells were again washed with PBS and then add purple fluorescent protein with primary antibodies. Then again slides were washed and put cover slip on the slides. Mouse IGg1 antibodies were used as a primary antibody.

RESULTS

In figure 2 from A to H explains the induced pluripotent stem cells which were not found to migrate into the uninjured adult retina after intra vitreal delivery. After the grafting of all layers of retina no human nuclear cells were observed in the retinal layer (C and D). The data in figure 2A and 2B explains the transplantation of the cells after 1 week. Then in 2C and 2D cells were engrafted after 2 weeks. All induced pluripotent stem cells not retained their EGFP expression. Histochemical labeling tells us that many human nuclear antigens are negative (figure 2A, 2B, 2C and 2D).

For the encouragement of chondroitinase we combine the chondroitinase with erythropoietin. This attempt modifies the inhibitory retinal environment. We calculate 19 to 35 cells $EGFP^+$-IPs cells/eye. These cells were not observed in the retina (figure 2A to 2I). It should be noted that this count is likely to be greatly underestimated, given that less than half of the induced pluripotent stem cells injected into the eye expressed the marker protein EGFP (Fig. 2).

The retina is exposed to EPO either they are in vitreous form or in subretinal space (figure 3). EFGP-positive cells were appeared in the left side of the retina. In the presence of EPO figure 3A is one of the surviving grafts of the retina. The cells appeared in the induced pluripotent stem cells and extend from ganglion to the outer nuclear layer (figure 3B). Co injection of chondroitinase ABC with IPs cells into the glaucomatous eye greatly enhanced the ability of the transplanted cells to invade the retina (figure 4E and 4G). Then cell goes to the retina and extends long.

The differentiation of induced pluripotent stem cells after their incorporation into the glaucomatous retina was also examined in this experiment. Immunohistochemical analysis showed that a large number of OPCs, positive for the nuclear marker Olig2, survived in the vitreous, proximal to the inner surface of the retina, R glaucomatous eye for up to 4 weeks.

DISCUSSION

Muller glial cells are the radial glial cells of the retina and have been shown to share a common phelytic lineage with retinal neurons and to derive from a common multipotent progenitor cells (Turner and Cepko, 1987). Retinal ganglion cell replacement (RGCR) is one of the best possible methods to restore the vision after glaucoma (Kerrigan-Baumrind et al., 2000; Quigley and Green, 1979). Stem cell transplantation has been shown to neuronal loss and also replace outer neuronal membrane (MacLaren et al., 2006; Gamm et al., 2007; Schraermeyer et al., 2001 and Qiu et al., 2005).

In the present study we examine that the IPs cells posses the stem cell like properties and a human muller cell lines which have the potential and ability to divide and regenerated. These cells are well explained in the treatment of many diseases in many experiments (Lawrence et al., 2007). We conclude that muller cells remain very easily in the eye and also respond to the environment.

As previously reported (Setzu et al., 2006), the throat also been shown to increase expression of MBP by oligodendritic precursor cells (OPCs) in

vivo and optionally oligodendritic precursor cells (OPC) mediated myelination of RGC axons normally unmyelinated retinal. We observed less oligodendritic precursor cells (OPC) differentiation into MBP - expressing cells in the retina than previously reported. It is not clear why this difference in myelin production was detected, but it may be due to the use of different breeds of rats which strain differences in the inflammatory response and protective autoimmunity has been documented.

Oligodendritic precursor cells (OPCs) were injected into the vitreous of both injured and glaucomatous eye was found to survive well in all experiments. In addition of these the grafted OPCs observed to spread across the inner membrane of retinal surface, which puts them for mediating the observed neuro protection. The number of grafted cells was lower in chronic graft which had been OPCs in vivo for 12 weeks, compared with acute graft in vivo for only 4 weeks.

We found that the retina of both normal and glaucomatous did not permit the integration of induced pluripotent stem cells because they are without extra cellular matrix modification. Even in the direct contact of retina IPs cells are unable to penetrate in the membrane (Banin et al., 2006; Hara et al., 2004 and Meyer et al., 2006). It was demonstrated that induced pluripotent stem cells and the two other similar cell lines could integrate into the retina of neonatal and injured adult rats and then they differentiated into different cells type (Lawrence et al., 2007 and Singhal et al., 2008).

We also find out the ability of muller cells to differentiate in the glaucomatous eye when cells were delivered in both cases either intravitreally or subretinally. The intra-vitreousroute is more reliable as compared to subretinal delivery because they fail in grafting. This experiment demonstrated that intravitreal injection was not without tribulations, as it was often complicated to place the cells precisely adjacent to the retina (Mooney et al., 2008).

In summary, we have demonstrated that the human-derived IPs progenitor cell line induced pluripotent stem cells is capable of surviving within the glaucomatous eye and of acquiring neural morphology upon intravitreal transplantation.

We also found that delivery of EPO and chondroitinase ability to migrate into the adult retina. With the particular environment retinal environment of chondroitinase facilitated the IPs cells into the mature retina.

CONCLUSION

The results explain that differentiating induced pluripotent stem (IPs) cells within the glaucomatous eye produced cells that expressed glial cell markers.

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There is no conflict of interest. All the authors contributed equally.

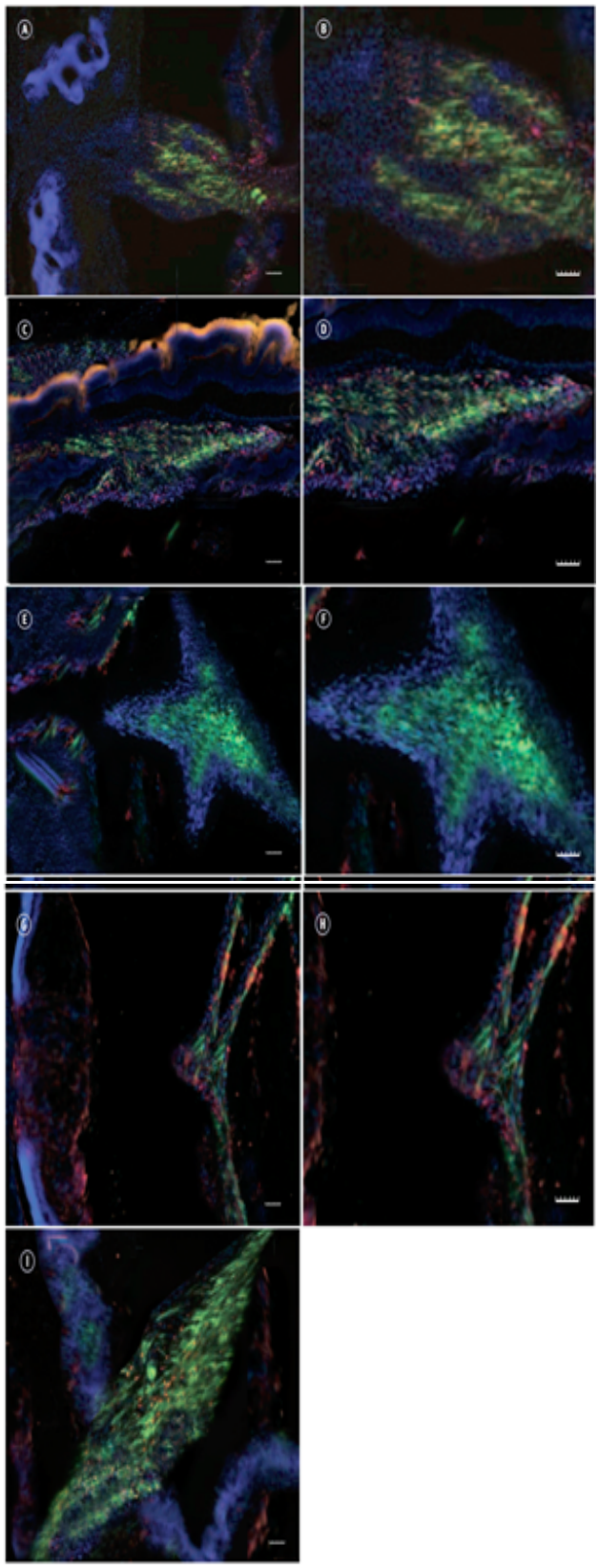

Figure 02: Survival and migration of IPs cells after transplantation into the eye (from A to H). In A and B cells were transplanted. In C cells were engrafted and C is magnified in D. In E and F the eyes were stained with DAP1 (purple).

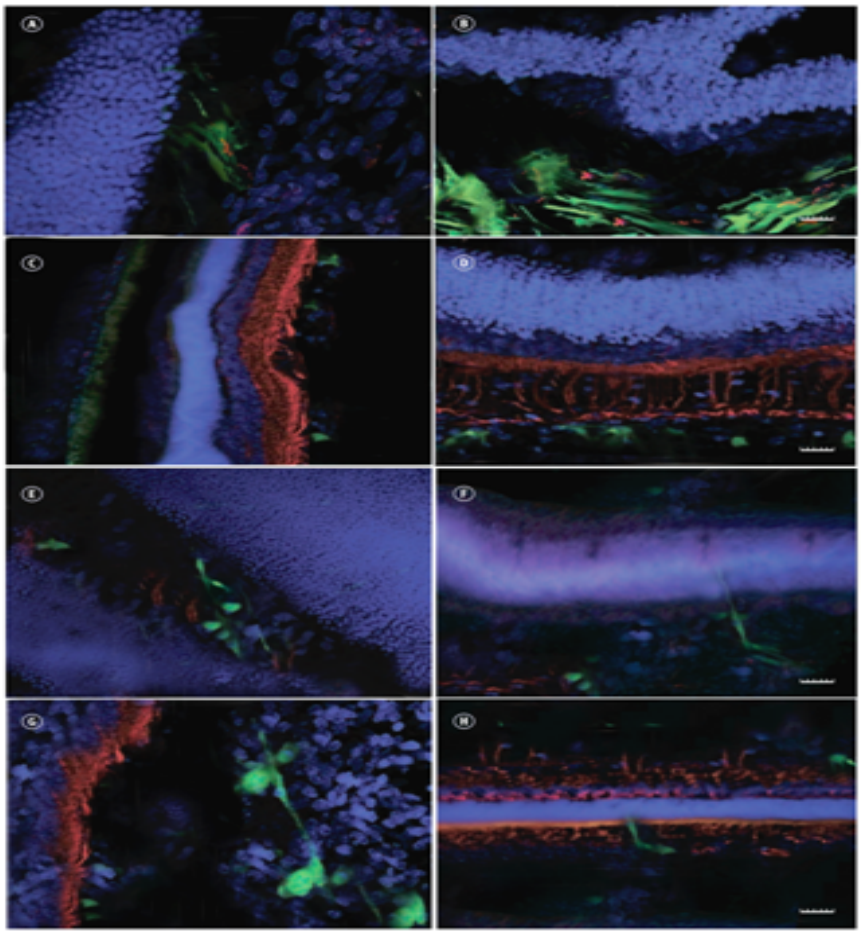

Figure 03: Facilitated IPs cells in the glaucomatous eye after transplantation of every 2 weeks in-vivo. In A to D intraocular (IO) injection of EPO and then cells migrate to retina. In D antibodies were used to detect DPA1 purple. Intraocular migration from E to F.Engrafted cells in G. Tissues were counterstained with DAP1 purple. Chondroitinase ABC activity was confirmed in H.

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