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Transplantation of reprogrammed embryonic stem cells improves visual function in a mouse model for Retinitis Pigmentosa

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Background

Specialized retinal cells called the retinal pigment epithelium maintain vision. Retinitis Pigmentosa is a inherited genetic disorder which results in loss of vision due to degeneration of both rod and cone photoreceptors (retinal cells) in the retina. Retinitis Pigmentosa is characterized by significant and unusual intraretinal pigmentation of the mid-peripheral retina. The death of cells in the retinal pigment epithelium (RPE) leads to blindness in many diseases, including age-related macular degeneration (AMD) and various forms of retinitis pigmentosa (RP). Worldwide, approximately 1.5 million people are afflicted with Retinitis Pigmentosa, and its incidence is expected to double by 2020. It is estimated that 30 percent of the population will have some form of macular degeneration by the time they reach the age of 75.

Hence, RPE loss accounts for a significant number of neurodegenerative diseases that severely impair activities of daily living and cause psychological depression. Cell transplantation into the human retina has the potential to restore lost vision and to provide treatment of advanced stages of retinal degeneration with significant RPE loss.¹⁻³ Replacement of damaged RPE in AMD is currently offered in many hospitals^{4,5} but the therapy is limited by a shortage of donor retinal pigment epithelium cells. Stem cells, in contrast, may provide improved cell transplantation material for future clinical practice. Recent studies suggest that ES cell - derived RPE cells are more similar to *in vivo* RPE cells than human RPE cell lines in morphology, gene expression, and immunohistochemical analysis.⁶ Although some differentiation protocols have derived RPE from ES cells of rodent⁷ and primate¹ origin, no studies have reported using these differentiated ES cells in treating diseased animals of the same species. It is essential to have results from animal disease models before applying this type of treatment to humans.

In this article authors successfully turned stem cells into retinal cells, and these retinal cells restored vision in a mouse model of retinitis pigmentosa. The transplanted cells not only looked like retinal cells, but they functioned like them, too. In this study,

sight was restored in one-fourth of the mice that received the stem cells. However, complications of benign tumors and retinal detachments were seen in some of the mice.

Study Design

The study was designed to demonstrate whether C2J ES (Yellow fluorescent protein (YFP)-labeled) cells will differentiate into RPE like cells and whether these cells improve retinal function in the *rd12* mouse (retinitis pigmentosa model). To accomplish this, authors differentiated the embryonic stem cells to RPE like cells. After differentiation, cells were immunostained for RPE markers and western blot was done. To observe morphological changes and test the expression of RPE markers, ES cells were differentiated *in vitro* with the differentiation medium for longer periods of time.

ES cell-derived RPE-like cells ($1.0 \times 10^3/1 \mu\text{L}$) were transplanted into the subretinal space of postnatal day 5 (P5) *rd12* mice (123 mice). To determine whether any rescue effects were due to surgery or feeder cells, authors grafted an additional three groups of *rd12* mice with phosphate-buffered saline (PBS), mitomycin-C treated PA6 feeders, and mitomycin-C treated undifferentiated mouse ES cells. Encouragingly, the ES cell-derived RPE-like cells expressed RPE markers, and the mice transplanted with these cells showed significant responses by electroretinogram (ERG) that did not occur in the control groups. To assess transplantation efficiency, retinas were examined by live imaging and Whole-mount eyecups were prepared for checking the survival and position of ES cells. ERG was done for functional measurements.

For checking that the differentiated cells can generate epithelial-like cells the authors examined the colonies and found that after approximately 23 days of differentiation, some cells exhibited an epithelial morphology. On average, 30% to 50% of the colonies formed epithelial-like cells after 3 weeks of differentiation. They also used RPE markers RPE65, bestrophin and ZO-1 to explore whether the ES cells could assume an RPE cell fate

after differentiation and performed immunoblots on extracts from the ES cells after 11 days of differentiation. After 11 days of differentiation, ES cell-derived RPE-like cells contained RPE65 protein, whereas bestrophin protein expression remained low. Human adult and fetal RPE were used as positive controls in the immunoblot analysis. These results indicate that ES cells could express some RPE markers after differentiation.

To further characterize the cell differentiation after transplantation, authors performed immunohistochemical studies to demonstrate the subretinal location of B6 ES graft at 2 weeks after surgery. Immunostaining with anti-RPE65 confirmed proper differentiation of grafted pigmented B6 cells and their subretinal location.

For testing the survival of YFP-labeled ES cells in the subretinal space they used the, *in vivo* live fluorescence microscopy and whole-mount eyecup imaging. The distribution of the YFP-positive cells is around the equator of the eyeball, which corresponds to the injection site. Cryosections of the same mount showed YFP-positive cell between the outer segment and RPE. Results from both *in vivo* live imaging and whole-mount eyecup show that the transplanted cells survive and can be located between the outer segment and the native RPE layer for as long as 7 months after transplantation. Authors also showed efficacy of stem-cell transplantation by ERGs. Mice showed increase ERG responses in the transplanted eyes compared with the control fellow eyes.

Implications

Nan-Kai Wang *et al* showed that this study provides the first evidence that ES cell-derived RPE-like cells restore visual function in a clinically relevant mouse model of retinal disease.

Stem-cell derivatives can be genetically marked by expression of fluorescent proteins and tracked in live animals by nonin-

vasive imaging. *In vitro* cultures showed that the PA6 stromal cell line can induce differentiated mouse embryonic stem (ES) cells to express RPE-specific markers, such as RPE65, bestrophin, and ZO-1. They showed efficacy of stem-cell transplantation by ERGs showing that retinal function can be restored indicating that stem-cell derivatives integrate functionally into degenerating *rd12* retina. Authors conclude that stem-cell transplantation has the potential to restore lost vision and provide treatment of advanced stages of RP and AMD featuring significant RPE loss.

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