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**Generation of retinal pigment epithelial cells from small molecules and OCT4-reprogrammed human induced pluripotent stem cells.**

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**Funding Grants:** Autologous Retinal Pigmented Epithelial Cells Derived from Induced Pluripotent Stem Cells for the Treatment of Atrophic Age Related Macular Degeneration

**Public Summary:**

Age related macular degeneration (AMD) is the leading cause of photoreceptor cell death and vision loss in the elderly. Photoreceptor cells are supported in the retina by retinal pigment epithelium (RPE) cells that perform multiple diverse tasks essential for photoreceptor cell function and survival. Dysfunction or death of RPE cells can initiate photoreceptor degeneration in AMD patients. Transplantation of healthy RPE cells into diseased retinas has been shown to slow progression of photoreceptor degeneration, and RPE cells can be readily generated from stem cells. Caution must be exercised to ensure that stem cell derived RPE resemble actual human RPE cells and function appropriately. In our lab we use induced pluripotent stem (iPS) cells that are generated from skin samples after "reprogramming" their gene expression profile to convert them into stem cells. Using this technology it may be possible to generate patient-matched stem cells and RPE grafts to minimize the risk of graft rejection. There is concern, however, that the reprogramming methods are not completely safe, especially since the genes introduced into the skin cells have been shown to induce cancer as well. Viruses are used to deliver the four reprogramming genes, and these introduce permanent changes into the host cell DNA. In this paper we introduce 1F-iPS-RPE cells that are derived from stem cells reprogrammed using only one of the four genes (to deliver the OCT4 gene using viruses) and small molecules that mediate only transient effects. The RPE cells generated using this approach strongly resemble actual RPE cells based on multiple examinations. First, the classic hexagonal shapes and black coloration of the cells typical of RPE cells are observed, and a known set of genes that RPE cells are known to express are synthesized by 1F-iPS-RPE. We also compared 1F-iPS-RPE and actual RPE using technology that is sensitive enough to detect the end products of multiple biochemical pathways. These experiments show that 1F-iPS-RPE and human RPE are remarkably (99.5%) similar. Perhaps most importantly, the transplanted cells integrate into the correct location in the retina in between the host RPE cells and can protect the photoreceptors from cell death in rats with dysfunctional RPE cells and photoreceptor degeneration. We also demonstrated that the transplanted 1F-iPS-RPE cells are functional since they ingest the tips of the photoreceptor cells that are sensitive to light damage. The surviving photoreceptor cells are also functional. We used novel technology to shine a beam of light over the region of the eye containing the RPE graft and measure the elicited light-responsiveness of the photoreceptors (electrical impulses) that could not be observed in regions in which no RPE cells were implanted. Therefore, we demonstrate in this study that 1F-iPS, which are reprogrammed using safer methods than conventional iPS cells, can be used to generate RPE cells that strongly resemble actual RPE and function appropriately when transplanted into animals. These results also provide encouragement that we are moving closer to developing an effective treatment for AMD.

**Scientific Abstract:**

Autologous retinal pigment epithelium (RPE) grafts derived from induced pluripotent stem cells (iPSCs) may be used to cure blinding diseases in which RPE dysfunction results in photoreceptor degeneration. Four, two, and one factor-derived iPS (4F-, 2F-, and 1F-iPSCs, respectively) were differentiated into fully functional cuboidal shaped pigmented cells in polarized monolayers that express RPE-specific markers. 1F-iPS-RPE strongly resemble primary human fetal RPE (hFRPE) based on proteomic and untargeted metabolomic analyses, and, utilizing novel in vivo imaging technology coupled with electroretinography, we demonstrate that 1F-iPS-RPE mediate anatomical and functional rescue of photoreceptors after transplantation in an animal model of RPE-mediated retinal degeneration. 1F-iPS-RPE cells were injected subretinally as a suspension and formed a monolayer dispersed between host RPE cells. Furthermore, 1F-iPS-RPE do not simply provide trophic support to rescue photoreceptors as previously speculated, but actually phagocytose photoreceptor outer segments in vivo and restore visual cycling (based on high-resolution mass spectrometry based detection of

recycled photoreceptor protein and lipid end products and electron microscopic analysis). Thus, 1F-iPS-RPE grafts may be superior to conventional iPS-RPE for clinical use since 1F-iPS-RPE closely resemble hFRPE, mediate anatomical and functional photoreceptor rescue in vivo and are generated using a reduced number of potentially oncogenic reprogramming factors.

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