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Original Article

Using induced pluripotent stem cell-derived conditional medium to attenuate the light-induced photodamaged retina of rats

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Abstract

Background: Light injury to photoreceptor cells and retinal pigment epithelium may lead to oxidative stress and irreversible degeneration of retina, especially degeneration of the high energy-demanded macula. The model of retinal photodamage could be applied to age-related macular degeneration and other degenerative retinal diseases for exploring new treatments. Based on broadly investigated induced pluripotent stem cells (iPSC) in the field of retinal degeneration, we aimed to clarify further how the interaction progresses between iPSC-conditional medium (CM) and light-damaged retina.

Methods: iPSCs were generated from murine embryonic fibroblasts of C57/B6 mice by retroviral transfection of three factors: Oct4, Sox2, and Klf4. Cytokine array was performed to analyze the components of CM. Sprague–Dawley rats receiving white light exposure to retina were viewed as an animal model of light injury. The rats were divided into four subgroups: light-injured rats receiving intravitreal injection of iPSC-CM, apoptotic iPSC-CM, or sodium phosphate buffer (PBS); and a control group without light damage. The electroretinography and thickness of outer nuclear layer were measured to document the therapeutic effects in each condition. Apoptosis arrays for detecting annexin V and caspase 3 were performed in the retinal tissues from each group.

Results: Murine embryonic fibroblasts were induced into iPSCs and expressed the marker genes similar to embryonic stem cells. These iPSCs can differentiate into Embryoid bodies (EBs), three germ layers *in vitro* and develop teratoma in severe combined immunodeficiency mice. The quantitative polymerase chain reaction of our iPSC-CM showed significantly elevated fibroblast growth factor-2, glial cell-derived neurotrophic factor, and insulin-like growth factor-binding proteins-1, -2, and -3. Compared to rats without photodamage, the light-injured rats receiving iPSC-CM had less reduction of outer nuclear layer thickness on Day 21 than other groups treated with either PBS or apoptotic iPSC-CM. In the same animal model, both a- and b-waves of electroretinography measurement in the group treated with iPSC-CM were significantly maintained compared to the control group and others with apoptotic iPSC-CM or PBS treatment. The apoptosis assay also demonstrated lower levels of annexin V and caspase 3 in the group with iPSC-CM treatment than in other groups presenting increasing apoptotic markers.

Conclusion: The conditional medium of iPSCs contains plenty of cytoprotective, immune-modulative and rescue chemicals, contributing to the maintenance of neuronal function and retinal layers in light-damaged retina compared with apoptotic iPSC-CM and PBS. The antiapoptotic effect of iPSC-CM also shows promise in restoring damaged neurons. This result demonstrates that iPSC-CM may serve as an alternative to cell therapy alone to treat retinal light damage and maintain functional and structural integrity of the retina.

Keywords: conditional medium; induced pluripotent stem cell; light damage of retina

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Retina degeneration generally leads to visual impairment. Its major causes are associated with old age, light injury, oxidative stress, and ocular trauma. Age-related macular degeneration (AMD) is the most important and the most challenging issue to deal with, because it involves the degeneration of retinal pigment epithelium (RPE) and photoreceptor cells. AMD is clinically divided into two categories: dry and wet types. There are several therapies for the treatment of wet AMD, the neovascular and more severe type, including using argon laser photocoagulation to treat degeneration-associated choroidal neovascularization (CNV). The therapeutic aim is to destroy the neovascular membrane by photocoagulation, but such treatment also destroys the overlying retina, with a resultant scotoma.

Photodynamic therapy for wet AMD has recently been approved in the USA. It utilizes a 10-minute intravenous infusion of verteporfin (a photosensitizing drug), followed by diode laser (689 nm) exposure to destroy the neovascularization. Vascular endothelial growth factor (VEGF) has been identified in recent decades as a pivotal treatment surrogate for CNV. However, the benefits of utilizing anti-VEGF agents to prevent CNV recurrence or occult pre-CNV are still under debate. Moreover, subfoveal fibrosis will also develop or progress in neovascular AMD even though patients have no significant subfoveal hemorrhage and have already been treated with anti-VEGF agents. The presentation of antifibrotic therapeutics may be beneficial in reducing the incidence of subretinal fibrosis[1]; however, none of the types of treatment mentioned above are the perfect solution to wet AMD.

Recent novel techniques have been able to generate induced pluripotent stem cells (iPSCs) from mouse and human adult somatic cells, such as fibroblasts, via the retrovirus-mediated transfection of four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4.[2−5] iPSCs have been further demonstrated to be indistinguishable from embryonic stem (ES) cells in morphology, proliferative abilities, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity.[4,5] iPSCs share the same features as ES cells and are capable of self-renewal and differentiation into three germ layers, offering potential for clinical cell therapies and tissue engineering.[2] Furthermore, iPSCs can be derived from the patient's own somatic cells to avoid potential immune response and ethical concerns. As a result, patient-derived iPSC therapy can provide better function in recipients than available ES cell lines can. Therefore, iPSCs are regarded as an excellent candidate for cell therapy and used in autologous transplantation without the risk of rejection.

Recent studies have shown that iPSCs present the capability of multilineage differentiation and further reduce the severity of cerebral ischemic injury in rats, endotoxin-induced acute lung injury and bleomycin-induced lung inflammation and fibrosis in mice.[6−9] We also found that both four-gene iPSCs, three-gene iPSCs, and their conditional medium (iPSC-CM) could reduce the level of inflammatory cytokines and chemokines in animals and thus decrease the inflammatory and fibrotic reaction.[8,9] Essentially, administration of iPSC-CM will not lead to the tumorigenesis that has been of greatest concern in stem cell therapy.

The treatment of light-damaged retina with iPSC-CM had not been commonly discussed before. In this study, our purpose was to demonstrate that iPSC-CM could suppress outer nuclear layer (ONL) thinning and recover impaired cellular function monitored by electroretinography (ERG) in light-injured murine retina. We hypothesized that the cytokines or chemokines in iPSC-CM might be beneficial to the light-damaged photoreceptors in the retina. Our findings may provide an alternative therapeutic strategy against light-induced retinal damage.

2. Methods

2.1. iPSCs cultured from murine embryonic fibroblasts

C57BL/6 mice were used in this study and all procedures involving animals were approved by the Animals Committee of the Taipei Veterans General Hospital. Our murine iPSCs were generated with only three introduced factors (Oct4/Sox2/Klf4) to avoid oncogene c-Myc (non-c-Myc iPSC). These iPSCs were generated from murine embryonic fibroblasts (MEFs) derived from 13.5-day-old embryos of C57/B6 mice. The reprogrammed iPSCs without c-Myc were transducted by retroviral vectors encoding three factors, Oct4, Sox2, and Klf4, as described previously.[10] Briefly, undifferentiated iPSCs were generally cultivated and expanded on mitotically inert MEFs (50,000 cells/cm^2) in six-well culture plates (BD Technology, Triangle Park, NC, USA) with 0.3% leukemia inhibitory factor in an iPSC medium consisting of Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum (FBS), 100 mM minimal essential medium nonessential amino acids, 0.55 mM 2-mercaptoethanol, and antibiotics. Every 3−4 days, colonies were separated with 0.2% collagenase IV, dissociated into single cells with 0.025% trypsin and 0.1% chicken serum in sodium phosphate buffer (PBS), and re-plated onto MEFs. The non-c-Myc iPSCs presented higher pluripotency and could be differentiated into three germ lines, including astroglial (neuro-ectodermal), osteogenic (mesodermal), and hepatocyte-like (endodermal) lineage cells.[10]

For induction of apoptosis, iPSCs were treated with 200 μM H_2O_2 for 18 hours. The definition of apoptosis was first based on a distinct sequence of morphologic features by microscopy (shrinkage of the cell and the nucleus), and further confirmed by caspase 3 activity and annexin V. After 6 hours of H_2O_2 treatment, the condition medium was collected for following experiment.

2.2. Light exposure and intravitreal treatment

All experiments were conducted in accordance with the Animal Care and Use Committee guidelines and in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Four-week-old, male Sprague−Dawley rats, each weighing 150−250 g, were raised in plastic cages in a