Subretinal Transplantation of Human Amniotic Membrane Mesenchymal Stem Cell Restores Retinal Function Loss on Light-induced Retinopathy

Chih-Cheng Chien¹², Yu-Jyun Cai¹³, Lu-Tai Tien¹, Yih-Jing Lee¹,*

ABSTRACT

Background and Purpose: Light-induced retinopathy is an animal model that mimics the photoreceptor apoptotic progress in retinal degeneration. This study examines the effects of human amniotic membrane mesenchymal stem cells (hAM-MSCs) on a light-induced animal model. Methods: Male Sprague-Dawley (SD) rats were exposed to 5000 to 7000 lux for 7 d and treated with hAM-MSCs. The functional changes of the retinas were monitored by electroretinogram (ERG). Frozen retinal sections were analyzed for histology and immunohistochemistry. Results: Severe damages presented, including a large number of apoptotic cells on the ONL, a decrease in the ONL thickness, and the reduction of ERG following 7 d of light exposure. Retinal structure and function were recovered following treatment with hAM-MSCs, and the grafted hAM-MSCs were differentiated into retinal pigment epithelium cells (RPE), photoreceptors, and Müller cells. The transplantation of the hAM-MSCs improved the retinal function and morphology, and the hAM-MSCs differentiated into retinal cells. Conclusion: These findings show that hAM-MSCs may be possibly used to treat retinal degenerative diseases.

Keywords: amniotic membrane mesenchymal stem cell; electroretinogram; light-induced retinopathy; cell apoptosis; retinal degeneration

INTRODUCTION

Age-related macular degeneration (AMD) is a retinal pathological disease that causes irreversible progressive damage to the macular, progressive loss of retinal cells, and visual disability or blindness. AMD typically occurs in people over 60 years old [1]; however, there are cases showing more and more people younger than 50 years old developing AMD in the recent years. Noell et al. demonstrated that laboratory rats’ retinas were influenced by
intense light [2]. Light exposure causes photoreceptors to undergo apoptosis in synchronization and to degenerate quickly [3]. This method has also been used in many studies: Numerous studies have used antioxidants delivery to prevent cell apoptosis [4-6], whereas other studies have shown that neurotrophic agents can provide neural protection from light-induced retinal damage [7-9]. In addition, some studies have shown that short-wavelength blue visible light induces retinal injury and may be a risk factor for age related macular degeneration [10]. Although these studies have proposed methods that can slow retinal degeneration, there has been no evidence showing that retinal degenerative diseases can be rescued or reversed.

Cellular-based therapy is the only treatment for retinal regeneration. This method resupplies damaged or faulty cells with neural progenitor cells or stem cells. Numerous studies have shown that stem cells can differentiate into neural-like cells or neurons and that they secrete some trophic factors, improving neurological function following the grafting of the stem cells into sites damaged by a central nervous system disease [11, 12]. Human amniotic membrane mesenchymal stem cells (hAM-MSCs) was supposed to be a novel source of stem cells and that were harvested from amnion [13], and exhibit the abilities of adipogenesis, osteogenesis, and chondrogenesis that were induced in vitro [14, 15]. Following treatment with a neuron induction medium, hAM-MSCs express neuron markers and astroglial markers [14, 16]. Sun et al. also demonstrated that hAM-MSC transplantation provided a cellular treatment for Amyotrophic lateral sclerosis in an animal model [17]. According to these studies, hAM-MSCs should be studied in the field of regenerative medicine. Therefore, we determined that there is potential for the application of hAM-MSCs to incurable retinal degenerative diseases.

**MATERIALS AND METHODS**

**Animals and light-induced retinopathy**

All of the animal experiments were conducted in accordance with the Statement for the Use of Animals in Ophthalmic and Vision Research of the Association for Research in Vision and Ophthalmology (ARVO) and were approved by the Institutional Animal Care and Use Committee (IACUC) of Fu-Jen Catholic University. Male Sprague-Dawley (SD) rats (175-200 g) were ordered from BioLASCO Technology (Taipei, Taiwan) and raised in a normal light cycle environment of 120 lux. During the exposure period, the SD rats were placed in cages containing 2 fluorescent tubes (27 W) of 5000 to 7000 lux in cyclic, cool white luminous for 7 d (12 h dark/12 h light) [18, 19]. Following light-induced damage, the SD rats were returned to a normal light cycle.

**Preparation of hAM-MSCs**

The cells were cultured in Dulbecco’s Modified Eagle Medium Low Glucose (DMEM/Low glucose, HyClone, Logan, Utah, USA) and Ham F-12 (HyClone, Logan, UT, USA; v/v = 1:1) with 20% fetal bovine serum (FBS; HyClone, Logan, UT, USA) and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA), and then basic fibroblast growth factor (bFGF; Millipore, Billerica, MA, USA) was added for a final concentration of 4 ng/ml in a culture dish that was 37°C in a humidified atmosphere of 5% CO₂. This study used hAM-
MSCs at passage 7. Prior to cell transplantation, the hAM-MSCs were labeled with bis-benzimide (Sigma, St. Louis, MO, USA). The labeled cells were collected the following day, washed, and resuspended in sterilized normal saline before subretinal injection.

**Subretinal transplantation**

The rats were anesthetized with Zoletil 50 (40 mg/kg, intraperitoneally; Virbac, France) one day after the light exposure completed for subretinal transplantation. Prior to subretinal injection, 0.5% proparacaine hydrochloride (Alcon, France) was dropped onto the rats’ corneas as a topical anesthesia, and their sclera were incised with a 30G needle (BD, Franklin Lakes, NJ, USA) on the temporal part of their eyes. One microliter of cell suspensions, containing $2 \times 10^5$ cells, was slowly injected into the subretinal space using a Hamilton syringe fitted with a 33G blunt-ended needle (Hamilton, Reno, NV, USA). The non-operated eyes of the rats were used as the control group ($n \geq 6$), the normal saline injected rats were grouped as the normal saline group ($n \geq 10$), and the rats that received hAM-MSCs were grouped as the AMMSC group ($n \geq 10$).

**Electroretinography**

The functional analyses of the retinas were performed using a scotopic electroretinogram (ERG). A RETIport ERG system (AcriVet, Germany) was used in this study, and the ERG recording methods were adapted from Bayer et al. [20] and Cheng et al. [19] with minor modifications. Following pre-adaptation to darkness, the SD rats were anesthetized with Zoletil 50 (40 mg/kg; Virbac, France) under a dim red light illumination. The average amplitudes of each a-wave and b-wave following light-induced retinal damage and were divided by amplitude of normal ERG and were compared to themselves as a denominator prior to 7 d of light exposure. The rats were examined by ERG as W0 following the induction of retinal damage for 7 d. Thereafter, the cells were injected.

**Histological study**

Retinal sections of 5 μm were cut on a cryostat and fixed with 4% formaldehyde (Showa, Japan) in PBS for 10 min and washed with PBS 3 times. The slides were stored at -80°C and prepared for subsequent experiments. Hematoxylin and Eosin (HE) staining was used to exhibit the retinal histology and thickness of the inner nuclear layer (INL) and outer nuclear layer (ONL). The method of estimating the retinal thickness was modified from those by LaVail et al. [21] and Cheng et al. [19] The thickness of the ONL and INL were measured in 9 sets of 5 measurements in each nasal and temporal hemisphere. The first set was made of 250 μm from the optic nerve, and subsequent sets were made of 250 μm and were measured more peripherally. An up-right microscope (Leica DM2500, Germany) with a digital image camera (Leica DFC4200, Germany) was used to examine retinal thickness and morphology.

**Apoptotic cell detection**

A TUNEL assay was performed with an apoptosis detection kit (In Situ Cell Death Detection Kit, Fluorescein, Roche, Germany) and was used to detect the breakdown of DNA strands in the apoptotic cells using fluorescence microscopy. The TUNEL-positive cells were counted in the entire area of each retinal section [22]. The images
were observed using fluorescent microscope (Leica DM2500, Germany) and a digital camera system (CoolSNAP EZ, Roper Scientific, Tucson, AZ, USA).

**Immunohistochemistry**

Following the fixation and washing, the retinal sections were blocked with 5% FBS (HyClone, Logan, Utah, USA) and washed with PBS. Each slide was incubated with a primary antibody, including mouse monoclonal anti-RPE65 (Chemicon, Temecula, CA, USA), rabbit polyclonal anti-rhodopsin (Chemicon, Temecula, CA, USA), rabbit polyclonal anti-Prox1 (Abcam, UK), rabbit polyclonal anti-syntaxin (1:200, SCBT, Santa Cruz, CA, USA), mouse monoclonal anti-Thy1 (SCBT, Santa Cruz, CA, USA), rabbit polyclonal anti-glial fibrillary acidic protein (GFAP, SCBT, Santa Cruz, CA, USA), goat polyclonal anti-glutamine synthetase (SCBT, Santa Cruz, CA, USA), rabbit polyclonal anti-SDF-1α (stromal cell-derived factor-1α, Torrey Pines Biolabs, Huston, TX, USA), and secondary antibodies, including Alexa Fluor 488 (IgG, Molecular probes, Eugene, OR, USA) and Alexa Fluor 555 (IgG, Molecular probes, Eugene, OR, USA). The fluorescent images were acquired using a fluorescent microscope (Leica DM2500, Germany) and a digital camera system (CoolSNAP EZ, Roper Scientific, Tucson, AZ, USA).

**Statistical analysis**

SPSS version 19 (IBM, Armonk, NY, USA) was used for data analyses. All data are shown as mean ± SEM. A Student’s t test and one-way ANOVA with Bonferroni multiple comparisons were used for statistical analyses, and $p < 0.05$ was considered a statistically significant difference.

**RESULTS**

**Light-induced photoreceptor degeneration**

This study was performed on rat retinas that were unexposed to light (Normal) and rats that were exposed to light for 7 d (W0) and continuously treated with or without hAM-MSCs for 1 wk. The results showed that a large number of the TUNEL-labeled apoptotic cells were detected on the ONL in the retinas that were exposed to light for 7 d (W0) (Figure. 1B), but not in the unexposed (normal) retinas (Figure. 1A). In addition, the images showed that the TUNEL-labeled apoptotic cells were decreased in hAM-MSC-treated (W1_AMMSC) (Figure. 1D) and untreated (W1_control) retinas (Figure. 1C) 1 wk after light exposure. The TUNEL-labeled apoptotic cells were counted in each retina, and the results are shown in Figure. 1E. The quantitative results showed that the apoptotic cells increased substantially following light exposure, but had decreased in the retinas of W1_AMMSC and W1_control (Figure. 1E). Statistical analyses showed a significant increase of apoptotic cells in the light-exposed retinas compared to normal retinas (Figure. 1E). These findings demonstrate that the light-induced retinopathy caused the photoreceptors proceed cell apoptosis on the ONL.

**Effects of hAM-MSCs on retinal morphology**

The frozen sections with HE staining were used to observe the structures of the retinas at different times. As shown in Figures. 2 and 3, a substantial decrease of the ONL thickness occurred,
but no apparent decrease of INL thickness was observed, compared to the normal retinas following light exposure (Figure. 2A, 2B, 3A, and 3B). Following exposure to light for 7 d, the ONL thickness of the control group and AMMSC group were collected and measured at 1 wk (Figure. 2C and 2D), 3 wk (Figure. 2E and 2F), and 5 wk (Figure. 2G and 2H) following light exposure. The layers of the ONL appeared to be thicker in the AMMSC group (Figure. 2C, 2E, and 2G) than those in the control group (Figure. 2D, 2F, and 2H). The quantitative ONL data (Figure. 4) revealed a significant differences on the ONL thicknesses between the control group and the hAM-MSC-treated retinas at 1 wk (Figure. 4A), 3 wk (Figure. 4B), and 5 wk (Figure. 4C) following exposure in certain areas. These results suggest that the transplantation of hAM-MSCs may reduce cell loss in ONL following long-term exposure.

**Effect of hAM-MSCs on retinal functions**

Prior to the light-induced damage, each rat received a normal ERG examination as the denominator, which was divided by the following ERG recording against itself and summarized as the recovering rate. Clear a- and b-waves were exhibited in the normal ERG (Figure. 5A), but the retinas of the light-induced damage group presented flat a- (10%) and b-wave (10%) forms (Figure. 5B), indicating that light exposure severely reduced the ERG function. The higher peaks of the b-wave were detected in hAM-MSC-treated retinas compared to the control retinas at 1 wk (Figure. 5C and 5D), 3 wk (Figure. 5E and 5F), and 5 wk (Figure. 5G and 5H) following the 7 d of light exposure. As shown in Figure. 6, a statistical analysis indicated no significant interaction between the control group (light-exposed without treatment) and AMMSC group in the a-wave at various times (Figure. 6A). Furthermore, there were no significant a-wave and b-wave differences between the control and normal saline-treated retinas groups (data not shown). However, the recovery rate of the b-wave showed a significant increase in the hAM-MSC-treated retinas compared to the control retinas (Figure. 6B). At the end of the experiment (5 wk after treatment), the recovery rate of the b-wave was 1.5 times ($p < 0.05$) higher in the AMMSC group than in the control group, although both groups showed gradual increases.

**Distribution of grafted hAM-MSCs into light-induced retinopathy**

The grafted transplanted hAM-MSCs are shown in Figure. 7. The fluorescent images were taken at the first, third, and fifth weeks following cell transplantation. The majority of the grafted hAM-MSCs were found in the ONL in the temporal hemisphere where the cells were injected at 1 wk (Figure. 7A), 3 wk (Figure. 7B), and 5 wk (Figure. 7C). As shown in Figure. 7C, some of the grafted hAM-MSCs migrated into the INL area in the temporal hemisphere 5 wk after the transplantations. Furthermore, a small number of transplanted cells were found in the nasal hemisphere (data not shown). This demonstrates that the hAM-MSCs were successfully transplanted into the subretinal space and had integrated into the retinal layers.

**Immunoreactivity of the grafted hAM-MSCs**

Following hAM-MSC treatment for 5 wk, the cell properties were recognized by different retinal cell makers using fluorescent immunohistoch-
istry, as shown in Figure. 8. Based on the images, some of the grafted hAM-MSCs expressed the retinal pigmented epithelial (RPE) cell marker RPE65 (arrows; Figure. 8A). An antibody of syntaxin was used as the amacrine cell marker, which was found colocalized with the transplanted hAM-MSCs at the edge of the inner plexiform layer (IPL) and ganglion cell layer (GCL; arrows in Figure. 8B). Some of the injected cells showed immunoreactivity to rhodopsin (arrows in Figure. 8C), which is the cell marker for photoreceptors. Figure 8D shows that the grafted hAM-MSCs located in the INL were labeled with Prox1 (arrow in Figure. 8D), which is a cell marker for horizontal and bipolar cells. Some of the injected cells represented GFAP (Figure. 8F) and glutamine synthetase (GS; arrows in Figure. 8G), which are cell markers for Müller cells. However, we did not discover any injected cells that expressed the ganglion cell marker Thy1 (Figure. 8E). In addition, we found some SDF-1α positive cells located near the ONL and RPE areas (Figure. 8H), and some of these SDF-1α positive cells were colocalized with the transplanted hAMS-MSCs (arrows in Figure. 8H). This finding suggests that the grafted cells may have been involved in the release of SDF1α for cell protection or repair.

**DISCUSSION**

This study found that the ONL thickness of the AMMSC group was thicker than that of the control group in some areas, and the b-wave of the ERG had a better recovery rate in the AMMSC group. However, the a-wave of the ERG did not show any significant differences between the two groups. ERGs are used in many clinical studies and laboratories to record the electrical potential of retinal neurons that are stimulated by a flash of light, and the ERG a-wave reflects the activation of the photoreceptors, the first event in visual transduction [23, 24]. Following this process, the ERG b-wave is activated and causes positive deflection, which reflects the activity of second-order neurons [24]. In addition, multiple photoreceptors converge on one bipolar cell, meaning that this bipolar cell is influenced by many photoreceptors through convergence. That is possibly why that increased ONL thickness did not proportionally response to the amplitudes of the a-wave but caused the higher recovery rate of the b-wave. Furthermore, numerous studies have determined the b-wave sensitivity curve by calculating the b-wave amplitude of the ERG plot against the luminance of the stimulating flash [25-27] with the b-wave amplitudes [6] as the retinal function. However, none of these studies measured the ERG a-wave. Many studies have shown that a reductive ERG b-wave is a sensitive index for ischemic injury, as demonstrated in humans and experimental models for retinal ischemia [28, 29]. Although our results showed that the b-wave did not completely recover, there was a substantially increased recovery rate of the b-wave (i.e., from 8% to 60%).

We presented the scattered TUNEL-positive cells on the ONL, which is composed of photoreceptor nuclei, and the results were consistent with those of previous studies [2, 30]. When the rats were removed from the damaging light after 1 wk, the frequencies of the TUNEL-positive profiles on the ONL fell in the SD and P23H rats [31]. A DNA laddering assay was used to detect apoptosis, and the DNA fragments were visible following light exposure, but disappeared after 4 d [32].
results were similar, and we observed apoptotic cells infrequently following exposure to light for 1 wk. However, some apoptotic cells were observed in the INL, although we could not define whether these apoptotic cells were caused by the light or by cellular regulation. In addition, we observed that the grafted hAM-MSCs were located not only on the ONL, but on the INL. We had assumed that the damaged sites would express some cytokines, such as Granulocyte colony-stimulating factor (GCSF), interleukin (IL)-1 and IL-3, or chemokine (e.g., IL-8 and SDF-1α) to promote stem cell mobilization [33].

In addition, we found that the expression of SDF-1α was upregulated in the damaged sites. Several studies have shown that SDF-1α and its receptor, CXCR4, are the key regulators for stem cells’ homing and recruitment. Following myocardial infarctions, SDF-1α is upregulated, inducing therapeutic stem cell homing to injured myocardial [34, 35]. Additional studies have shown that over-expression of SDF-1α in mesenchymal stem cells can increase the production of VEGF to promote angiogenesis, which can prevent progressive heart dysfunction following myocardial infarctions [36, 37]. Stumm et al. elucidated the cerebral functions of SDF-1/CXCR4 in adults and found an increase in the expression of SDF-1α near the infarcted area in the focal cerebral ischemia [38]. The mediation of the migration of bone marrow stromal cells (BMSC) following ischemic brain damage by SDF-1α has been demonstrated [39]. SDF-1α mRNA and various proteins have been localized in the ischemic penumbra, and pretreatment with the CXCR4 antagonist can prevent the migration of BMSC to the infarcted site [39]. Therefore, the migration of transplanted cells was affected by the local production of SDF-1α and the expression of CXCR4 on the BMSC surface [39]. Similar results were found on the transplantation of human umbilical cord-derived stromal cells (HUCMSCs) to the stroke model. For example, SDF-1α cells were upregulated in lesions and the grafted HUCMSCs expressing CXCR4 positively migrated to the ischemic boundary hemisphere, possibly indicating that cerebro-endothelial SDF-1α is a chemo-attractant for HUCMSCs [11]. The SDF-1α/CXCR4 showed the same important characteristics for mediating the migration of neural progenitor cells to the sites of ischemic damaged neurons [40].

Previous studies have observed that the SDF-1α levels were raised in the vitreous humor of ischemic ocular diseases, such as proliferative retinopathy and retinopathy of prematurity [41-43]. In addition, patients with retinal detachment (RD) showed higher SDF-1α levels in the vitreous humor compared to naïve patients, and endogenous SDF-1α is tissue-protective in RD [41]. Curcumin interferes in SDF-1α/CXCR4 signaling, inhibiting human retinal endothelial cell (HREC) migration. One study found that treatment with SDF-1α increased the HREC migration dose dependently, and in contrast, HREC migration was reduced in a dose-dependent manner by inhibiting CXCR4 expression. Curcumin blocks Ca²⁺ and downregulates the SDF-1α-induced expression of CXCR4 and PI3-K/AKT signals [44].

Many studies have shown that grafted stem cells can differentiate into retinal cells in injured retinas. In a previous study, bone marrow-derived stem cells (BMCs) were transplanted into the vitreous space in injured retinas, and the cells presented the cell markers of GFAP, calbindin, rhodopsin, and vimentin [45]. Subretinal transplantation of
BMCs was also reported as inhibiting photoreceptor apoptosis and slow down retinal damage in light-damaged eyes [46]. These cells were found express bFGF and BDNF in the in vitro study [46]. Following the transplantation of human cord blood cells (HCBCs) into the subretinal spaces of severely combined immunodeficiency (SCID) mice, the cells expressed human MAP2, human neuron specific enolase (NSE), β-τubulin, and rhodopsin [47]. However, these studies did not examine retinal physiological functions. In our immunohistochemistry study, we showed that the grafted cells differentiated into various retinal cell types, such as rod photoreceptors, RPE cells, and Müller cells. Furthermore, we proved that transplanting hAM-MSCs can restore retinal functions by using ERGs. Although the ganglion cell marker Thy1 was not colocalized with the grafted cells in our results, this is compatible with the results of the cell distribution, in that the grafted-hAM-MSCs were not observed on the GCL.

In conclusion, the proposed retinal degenerative animal model caused by 7 d of light exposure resulted in severe photoreceptor degeneration, a reduction in ONL thickness, and decreases in the amplitudes of the a-wave and b-wave of ERG. Following the transplantation of the hAM-MSCs, the structures and functions of the treated retinas showed better recovery than those without treatment. Furthermore, we observed that the grafted hAM-MSCs integrated into the retinal cell layers and expressed several retinal cell markers. Therefore, the transplantation of hAM-MSCs can improve retinal function and morphology. In addition, hAM-MSCs can differentiate into retinal cells. These findings suggest that hAM-MSCs are a possible therapy for retinal degenerative diseases.

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REFERENCES


Figure 1  Apoptotic retinal cells were detected by TUNEL assay. Microimages showing the TUNEL-labeled apoptotic cells (arrows) in a normal retina (A), a retina exposed to light for 7 d (W0) (B), an untreated retina (W1_control) (C), and an hAM-MSC-treated retina (W1_AMMSC) (D) 1 wk after the 7-day light exposure process. The apoptotic cells were counted in each preparation (E) and dramatically increased in the retinas exposed to light for 7 d. Data are shown as mean ± SEM (n=12); One-way ANOVA with Bonferroni multiple comparisons was used for statistical analysis. * indicates $p < 0.05$ compared, to normal retinae; # indicates $p < 0.05$ compared to the 7-day light-exposed retinas. Scale bar = 100 μm.
Figure 2  Histological images of retinas at different conditions. Microphotographs show a normal retina (A), a light-induced retina (W0) (B), and hAM-MSC-treated retinas and untreated retinas at 1 wk (C, D), 3 wk (E, F), and 5 wk (D, H) following exposure to light for 7 d. A high number of cells on the ONL decreased following light exposure for 7 d compared to normal retinas (A and B). The ONLs of the hAM-MSC-treated retinas were thicker than those of untreated retinas (C to H) at the same period. Scale bar = 100 μm.
Figure 3  Quantification of the retinal ONL and INL thicknesses in normal and 7-d light-exposed retinas. The thickness of the ONL decreased noticeably (A), but there was no change in the INL thickness (B) compared to normal retinas following light exposure for 7 d. Data are shown as mean ± SEM; Student’s t test was used for statistical analysis. * indicates $p < 0.05$. 
Figure 4  Quantification of the retinal ONL thicknesses in various groups at different times. The ONL thicknesses of the hAM-MSC-treated and untreated control retinas were measured at 1 wk (A), 3 wk (B), and 5 wk (C) following 7 d of light exposure. The retinas treated with hAM-MSCs preserved the ONL cells better than did those without treatment at defined points. Data are shown as mean ± SEM; Student’s t test was used for statistical analysis. * indicates \( p < 0.05 \).
Figure 5  Examples of the ERG recording. The retinal functions of an adult normal rat (A), an adult rat exposed to light for 7 d (W0) (B), adult rats receiving hAM-MSCs retinal transplants at 1 wk (D), 3 wk (F), and 5 wk (H) were exhibited. The eyes of the rats in the control group without hAM-MSCs treatment at 1 wk (C), 3 wk (E), and 5 wk (G).
Figure 6  Quantification of the ERG recording. Amplitude of a-wave (A) and b-wave (B) is presented as recovery rates ($n \geq 6$). The results show that the recovery rate of the b-wave in the AMMSC group was substantially higher than that in the control group following treatment for 1 wk, 3 wk, and 5 wk. Data are shown as mean ± SEM; Student’s $t$ test was used for statistical analysis. * indicates $p < 0.05$. 
Figure 7  Distribution of the transplanted cells in the retinas. Following exposure to light for 7 d, the hAM-MSCs were transplanted into retinas at the subretinal space. The grafted cells that were labeled with bis-benzimide were observed as blue fluorescence, as shown in the images for 1 wk (A), 3 wk (B), and 5 wk (C). The majority of the transplanted hAM-MSCs were located on the ONL (arrows) in the retinas (A, B, and C), and some cells migrated to the INL (arrowheads) 5 wk after transplantation (C). Scale bar = 100 μm.
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Figure 8 Images from the immunohistochemical study of grafted hAM-MSCs. Different retinal cell markers were used to detect possible expressions of the transplanted hAM-MSCs 5 wk after transplantation: RPE cell marker RPE65 (A), amacrine cell marker syntaxin (B), photoreceptor cell marker rhodopsin (C), horizontal and bipolar cell marker Prox1 (D), ganglion cell marker Thy1 (E), Müller cell markers GFAP (F), glutamine synthetase (G), and SDF-1α (H). Scale bar = 50 μm.
中文摘要

背景目的:以光照诱发视网膜退化病变的动物模式来探讨视网膜感光细胞凋亡的变化，并探讨视网膜下腔移植羊膜间质干细胞可能的治疗或视网膜保护功效。

实验方法:实验以SD大鼠为实验动物，饲养于光照照度5000到7000 lux，光暗循环各12小时的环境下7天，用以诱发视网膜退化动物模式。光照完成后，部分动物进行视网膜下腔移植人类羊膜间质干细胞，另一部分动物同部位注射生理食盐水作为对照组，并以视网膜电位图、视网膜组织切片、以及免疫萤光染色等方式进行不同组别之研究。

结果讨论:实验结果显示，光照一周后视网膜感光细胞有明显减少（凋亡）的现象，而视网膜电位图亦显示视网膜功能已经明显退化。经过视网膜下腔移植人类羊膜间质干细胞之后，三至五週後可以见到移植的羊膜间质干细胞已经嵌入被移植动物的视网膜组之中，並且减少感光细胞的凋亡。这些接受移植的动物在视网膜电位图的表现也比对照组顯示較佳的视网膜功能。藉由免疫螢光染色的技術更进一步发现，这些移植的羊膜间质干细胞逐渐分化为视网膜的色素上皮细胞（RPE）、感光细胞（photoreceptor）、以及移動膠細胞（Müller cells）。

结论:研究结果显示移植人类间质干细胞可以修复因长期光照造成受损的视网膜感光细胞并且光照后维持视网膜功能，期望研究结果可以做为临床治疗视网膜退化疾病之参考。

关键字:羊膜间质干细胞；视网膜电位图；光照诱发视网膜病变；细胞凋亡；视网膜退化疾病

1 腦神經學系
2 綜合臨床醫學系
3 腦神經學研究所
投稿日期:2016年10月21日
接受日期:2016年12月20日
通讯作者:李憶菁
电子邮件:yjlee@mail.fju.edu.tw