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PRINCIPAL INVESTIGATOR: Shaomei Wang

CONTRACTING ORGANIZATION:  Cedars-Sinai Medical Center
Los Angeles, CA 90048

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**Non-Invasive Cell-Based Therapy for Traumatic Optic Neuropathy**

**Author(s):** Shaomei Wang  
E-Mail: shaomei.wang@cshs.org

**Performing Organization Name(s) and Address(es):**  
Cedars-Sinai Medical Center  
8700 Beverly Blvd. AHSP, 8fl  
Los Angeles, CA 90048

**Sponsoring/Monitoring Agency Name(s) and Address(es):**  
U.S. Army Medical Research and Materiel Command  
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14. ABSTRACT

1. Traumatic optical neuropathy (TON) results from trauma to optic nerve by head and eye injuries to both military and civilian population such as accidents, and blast related combat trauma. TON leads to irreversible blindness and represent a major public health burden with both economical and social impacts. Unfortunately, treatment is still rather limited. A large body of evidence indicates that TON affects optic nerve and its target neurons in the central nervous system, which provide vital retrograde trophic support to optic nerve. We hypothesize that systemic administration of bone marrow derived mesenchymal stem cells (MSC) to treat traumatic optic neuropathy (TON) will preserve/repair optic nerve, stabilize the unstable environment due to trauma and promote RGC regeneration and outgrowth by promoting the release of paracrine and autocrine mediators; induced Schwann cells from MSC (M-Sch) will repair the damaged RGC by remyelinating and providing multiple trophic factors. Previous studies have shown that activation in retinoic acid (RA) signalling triggers neurite outgrowth in adult mice. Here we found that intravitreal injection of retinoid X receptor agonist SR11237 not only preserved RGCs, and promoted RGC axon outgrowth at both 8 days and 14 days after TON. We have used Long Evan (LE) rats as a model for TON, MSC were isolated from LE rats, M-Sch were induced from MSC. Our main findings: (a) Using our modified forceps, a reliable and reproducible TON model was created. (b) Rat MSC and M-Sch were reliable produced for experiments. (c) Systemic administration of MSCs significantly preserved retinal ganglion cell survival after TON. (d) Systemic administration of MSCs also promote limited RGC axons regeneration. (e) Intravitreal injection of retinoid X receptor agonist SR11237 also protect RGC survival after TON and promote RGC regeneration. (f) Systemic administration of MSCs induced up expression of trophic factors in the retina (CNTF, BDNF, bFGF). (g) Intravitreal injection of retinoid X receptor agonist SR11237 combined with systemic administration of MSCs promote RGC survival and axons regeneration after TON.

This study showed that systemic administration of MSC could significantly protect retinal ganglion cells after TON. Intravitreal injection of SR11237 protect RGCs after TON and promote RGC axon regeneration. We also showed that up-regulation of trophic factors in the retina after MSC injection into TON model, which is, at least in part the mechanism of MSCs in protecting RGCs after injury.

15. SUBJECT TERMS

None Listed

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INTRODUCTION

Traumatic optical neuropathy (TON) results from trauma to optic nerve by head and eye injuries to both military and civilian population such as accidents, and blast related combat trauma [1-3]. In a military report, 82% of severe eye injuries were caused by blast and blast fragmentation. TON leads to irreversible blindness and represent a major public health burden with both economical and social impacts. Unfortunately, treatment is still rather limited. Cytokine-mediated neuroprotection has been repeatedly demonstrated, and reliably reproduced, in multiple animal models with a range of optic nerve injury conditions [4-6] and block neuronal cell death in an excitotoxicity animal model [7-9]. A significant challenge to clinical implementation of this work is that cytokines are rapidly degraded by endogenous proteases. So the effect is short lasting. A direct and reliable approach to stem cell-mediated neuroprotection is a rational approach. A large body of evidence indicates that TON affects optic nerve and its target neurons in the central nervous system, which provide vital retrograde trophic support to optic nerve [10-12]. As an alternative approach, we propose a non-invasive, systemic delivery of stem cells to optic nerve and related target neurons in the brain. The systemic administration of stem cells offers substantial advantages over local delivery. These cells can exert therapeutic effects over the injured optic nerve and its targeted neurons in the brain, and multiple injections can be performed if needed. Others have successfully used the intravenous administration of MSC for treating stroke, cerebral ischemia, brain injury and myocardial infarction [13-15]. Based on the extensive experience with both MSC and M-Sch as therapies for regenerative and degenerative medicine, this study will determine whether it is realistic to transfer this treatment to the clinical setting.

BODY

1. Animal models: Long Evan rats are used throughout the study. Traumatic axonal injury (TAI) model was first created with a 60-g Yasargil aneurysm clip (Aesculap AG &C0, Tutlingen, Germany, arrow in image A) according to published protocol (16). However, we found it is impossible to use the Yasargil aneurysm clip, since it cannot perform ‘dissecting’, which is critical for free blood vessels from optic nerve bundle. We made a special clip by modifying a fine forceps (see image B). A reliable TAI model is created by using this modified forceps.
2. Isolation rat bone marrow derived mesenchymal stem cells (MSC, see image A); induction of Schwann cells from MSC (M-Sch, see image B) and MSC were purified with BD IMagnet combine with FACS analysis.

Isolation MSC from Long Evans rats using our published protocols (17), Image A showed MSC at passage 0.

Induction of Schwann cells from MSC according to published protocol (18-19). Image B showed antibody against S100 staining of Schwann cells induced from MSC. Graph C showed FACS analysis after CD54/CD90 selection.
3. RGC axon regrowth after injection of SR11237 following TON

Immediately after ONC, retinoid X receptor agonist SR11237 (4µM) was injected vitreously. CTb (green) was injected 3 days before animal euthanization. Optic nerves were collected at 7 and 14 days after ONC and stained with growth-associated protein 43 (GAP-43, red), counterstained with DAPI (blue). These images show that ONC without treatment, there is hardly regeneration of RGC axons; while in SR11237 treated eyes; there is regrowth of RGC axons at 7 and 14 days after ONC.

4. Inner retinal changes after intravitreal injection of SR11237 following TON

TON also affects photoreceptors: one week after TON, photoreceptor outer segments were obviously reduced in length even after retinoid X receptor agonist treatment compared with wild type untreated control.
Retinal sections were stained with antibody against cone arrestin after injection of SR11237 following ONC. At one week, cone morphology is preserved compared with normal control; while in untreated retina, both inner and outer segments were further reduced in length. Further examination showed that RGC dendrites as revealed by βIII-tubulin (green) are also preserved.
Retinal sections from SR11237 treated after ONC showed that there is up-regulation of β1 integrin (red) in the inner nuclear and RGC layers compared with normal untreated control.

5. Trophic factor expression after intravenous injection of MSCs following TON

![Graph showing bFGF mRNA expression](image)
Total RNA was extracted from retinas 7 days after TON following intravenous injection of 2 millions of MSCs; control samples were from untreated and TON without intravenous injection of MSCs. We found the expression of CNFT and bFGF was significantly higher in retina receiving MSC treatment compared with controls.
6. Functional evaluation

**Electroretinogram (ERG)** measure full-field retinal potential from the cornea. Under scotopic conditions, a-wave is generated by photoreceptor phototransduction; b-wave is mainly generated by depolarization of ON-bipolar cells and Müller cells. The Scotopic threshold responses (STR) are created from the inner most retina, where the RGC bodies are located.

Our study showed that at one week after ONC without treatment, both a- and b-waves reduced compared with untreated wild type rats. Further study with measuring STR did not detect significantly difference between TAI and TAI followed by MSC treatment.

**Luminance threshold recording (LTR) from the superior colliculus**

The functional state of the retina was evaluated by recording the multi-neuronal responses in multiple (16–18) microelectrode penetrations into the unilateral superior colliculus (SC) of anesthetized rats. At each recording site, the receptive field was located by presenting flashes of the light spot of 3° in diameter. Response luminance threshold was then measured and defined as a minimal luminance of the stimulating light spot eliciting criterion multi-unit response (of amplitude twice of the level of the background activity). This procedure results in a map of focal luminance thresholds over the whole visual field of the eye contralateral to the tested SC. Based on these recordings, the cumulative curve of the luminance thresholds across the retina was calculated, which showed the percent of retinal area (y-axis) where the visual thresholds were less than the values indicated at the x-axis. Our LTR showed that there was no signal recorded after optic nerve crush, indicating the integrity of retina is needed for luminance threshold recording. We did not perform LTR during the second year.

**KEY RESEARCH ACCOMPLISHMENTS**

- Reliable create TAI rat model by using our modified forceps
- Reliable isolate rat MSC and induction of Schwann cells from MSC (M-Sch)
- Worked out a new protocol for MSC purification with BD IMagent combine FACS analysis
- Reliable anterogradely label retinal ganglion cells and axons by injecting CTB into vitreous cavity; prepare for retinal whole mount
- Reliable retrogradely label retinal ganglion cells by applying fluorogold onto the superior colliculus
- Worked out protocol for quantifying retinal ganglion cells on retinal whole mount preparation
- Intravenous administration of MSC protect retinal ganglion cells after TAI and promote axons regeneration
- Intravenous injection of retinoid x receptor agonist protects RGCs and promote RGC axon regrowth after TON
- Intravenous injection of retinoid x receptor agonist also preserve inner retina
- Trophic factors-bFGF, CNTF and BDNF were up-regulated after TON following intravenous injection of MSCs
- Applying M-Sch to optic nerve crush site preserve retinal ganglion cells after TAI
- Luminance threshold recordings from the superior colliculus failed to detect retinal
activities after TAI.
• Optokinetic response provides non-invasive measurement for TAI model

REPORTABLE OUTCOMES:

The research was presented at ARVO, 2014, Orlando, FL.

Data collect is finished, manuscript is in preparation.

• CONCLUSION: We have reliably created rat model for TAI; reliably isolated MSC and induction of Schwann cells. We have found that non-invasive administration of MSC can protect retinal ganglion cells after TAI and local administration Schwann cells derived from MSC also protect retina ganglion cells. Systemic administration of MSC promotes axon regeneration, however axon regrowth is rather limited. To evaluate retinal function after TAI and intervention, optokinetic response also provides valuable indication of retinal function; and luminance threshold recording for the superior colliculus fails to record any retinal activities after TAI. Intravenous injection of retinoid X receptor agonist protects RGC survival and promotes RGC axon regrowth after TON. Trophic factors bFGF, CNTF and BDNF were up-regulated after intravenous injection of MSCs following TON. Future study: long-term evaluation of retinal ganglion cell protection and axon regeneration after intervention; new regents that have approved to promote axon regeneration combine with systemic administration of MSCs.

Future study

Current study showed that intravenous injection of MSCs can protect RGCs and preserve retinal integrity after optic nerve crush in rat model. Intravitreal injection of retinoid X receptor agonist promotes RGC survival and RGC axon regrowth after optic nerve crush. However, the RGC axon regrowth is rather limited. Future study will use optic nerve injury model that is mild (without optic nerve transection), such as trauma. The critical step to preserve vision after optic nerve injury is to protect RGCs from dying even for 1-2 weeks, which will provide a window for other therapeutic intervention. Combined therapies are needed to preserve RGCs and promote its axons regrowth after optic nerve injury at the same time.
REFERENCES


**APPENDICES:** PI's biosketch

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Principal investigator (Wang, Shaomei)

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**BIOGRAPHICAL SKETCH**

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<th>NAME</th>
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<tr>
<td>Shaomei Wang</td>
<td>Associate Professor</td>
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| eRA COMMONS USER NAME | Wangsha                     |

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<tr>
<td>Jinzhou Medical College, PR China</td>
<td>B.M. (M.D)</td>
<td>1984</td>
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<td>Chinese Medical University, PR China</td>
<td>Masters</td>
<td>1987</td>
<td>Neuroscience</td>
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<tr>
<td>University of Sheffield, UK</td>
<td>Ph.D.</td>
<td>1997</td>
<td>Visual neuroscience</td>
</tr>
<tr>
<td>Institute of Ophthalmology, UCL, London</td>
<td>Post-doc</td>
<td>2001</td>
<td>Cell-based therapy</td>
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A. Personal Statement

Our laboratory has a long history of applying cell-based therapy for retinal degeneration. We have explored the efficacy of a range of different cell types (i) cells to replace defective retinal pigment epithelial (RPE) cells such as human embryonic stem cell derived –RPE cells, (ii) cells that appear to function by releasing growth factors such as peripheral nerve ensheathing cells (Schwann cells) or cells genetically modified to release growth factors and (iv) cells with multiple functions such as stem cells and progenitors. Our studies have provided the pre clinical data for three prominent first in man human clinical trials for retinal degeneration using either human embryonic stem cells (Advanced Cellular Therapeutics) or adult mesenchymal stem cells (Johnson and Johnson) or central nervous system derived neural stem cells (StemCell inc). Recently, my laboratory has pioneered a new approach to treatment involving systemic administration of mesenchymal stem cells and shown extensive morphological and functional preservation in rodent models of retinal disease. I was recruited to the Cedars-Sinai Regenerative Medicine Institute where we are collaborating with the director, Dr Clive Svendsen who has a long history of using stem cells to model and treat diseases of the CNS, Dr Alexander Ljubimov, director in eye program, and immunologists with an interest in transplantation. I will continue to work with our long-term collaborators Dr Gamm (U of Wisconsin) using retinal progenitors/stem cells to limit retinal degeneration. Our preclinical studies will be focused on the efficacy, long-term survival of donor cells, mechanism of action and immunological responses after cell-based therapy. The object of our translational research program is to treat retinal degeneration and optic nerve repair with cellular therapy.

B. Positions and Honors.

Positions and Employment

2012-prosent  Associate professor, Cedars-Sinai Regenerative Medicine Institute, LA, CA
2006 - 2011  Assistant Professor, Casey Eye Institute, OHSU, Portland, USA
2005- 2006  Assistant Professor, Moran Eye Center, Utah, USA
2001 - 2005  Senior lab specialists, Moran Eye Center, Utah, USA
1997 - 2001  Post-doctoral fellow, Institute of Ophthalmology, UK
1994 - 1997  PhD student, University of Sheffield, UK.
1991 - 1993  Visiting Scholar, University of Sheffield (supported by British Council).

Honors

1984-1987  Graduate studentship, Jinzhou Medical college, PR China
1991-92  British Council and Chinese government award (visiting scholar to University of Sheffield, UK)
1992  Overseas Research scholarship
2004  Permanent US residency awarded in the US National Interest on the basis of outstanding researcher
2010  Paper was selected as Paper of the Month (March) at OHSU med school.
2014  First prize Faculty elevator pitch at Regenerative Medicine Institute retreat

**Other Experience and Professional Memberships**

- Member of ARVO’s Global Presence Pillar Steering committee
- Association for Research in Vision and Ophthalmology
- Society for Neuroscience
- Editorial member of Transplantation & technology and research
- Editorial member of International Journal of Ophthalmology
- Investigative Ophthalmology and Visual Science
- Experimental Eye Research
- Vision Research
- Current Eye Research
- Expert Reviews
- Stem cells international
- Plus ONE

**B. Selected peer-reviewed publications**


Yuchun Tsai†, Bin Lu†, Benjamin Bakondi, Sergey Girman, Anais Sahabian, Dhruv Sareen, Clive N. Svendsen, Shaomei Wang. Human iPSC-derived neural progenitors preserve vision in an AMD-like model. Stem Cells, 2015, April

Abstracts
Bin Lu; YuChun Tsai; Grazyna Adamus; Sergey Girman; Lin Shen; David M. Gamm; Catherine W. Morgans; Brandon Shelley; Clive Svendsen; Shaomei Wang. Effect of Repeated Delivery of Neural Progenitors on Vision Preservation in RCS Rats 2013, ARVO, A0030.

Shaomei Wang; YuChun Tsai; Sergey Girman; Lin Shen; Catherine W. Morgans; Grazyna Adamus; Weidong Xiong; Vaithi Arumugaswami; Clive Svendsen; Bin Lu. Manipulating MSC Homing To Enhance Its Efficacy in Preserving Vision 2013, ARVO, B0194.

YuChun Tsai; Bin Lu; Sergey Girman; Clive Svendsen; Robert M. Cohen; Shaomei Wang. Ocular changes in a rat model of Alzheimer’s Disease 2013, ARVO, D0232.

Jing Yang, Geoffrey P. Lewis; Bin Lu; Nikolay Turovets; Gabriel Luna; Sergey Girman; Gerhard Bauer; Steven K. Fisher; Shaomei Wang; Henry J. Klassen. Translational development of human retinal progenitor cells for treatment of retinitis pigmentosa 2013, ARVO, A0106.

Benjamin Bakondi; YuChun Tsai; Bin Lu; Sergey Girman; Lin Shen; Melissa K. Jones; Shaomei Wang. Is Mesenchymal Stem Cell Homing To The Injured Retina Required For Visual Preservation In The RCS Rat? 2014, ARVO, A0133.

YuChun Tsai; Bin Lu; Sergey Girman; Benjamin Bakondi; Melissa K. Jones; Anais Sahabian; Dhruv Sareen; Clive Svendsen; Shaomei Wang. Human iPSC-derived neural progenitor cells preserve vision in a rat retinal degeneration model.2014, ARVO, D0037.

Melissa K. Jones; Bin Lu; Sergey Girman; YuChun Tsai; Benjamin Bakondi; Brandon Shelley; Alexander V. Ljubimov; Clive Svendsen; Shaomei Wang. In vivo study of neural progenitors after injection into a retinal degeneration rodent model. 2014, ARVO, C0172.

Shaomei Wang; YuChun Tsai; Benjamin Bakondi; Sergey Girman; Lin Shen; Bin Lu. Systemic administration of MSCs preserves RGC survival after optic nerve crush. 2014. ARVO, B0139.

C. Current Grant Support

1RO1EY020488 Wang (PI)
09/01/2011-08/31/2016
NIH/NEI
Development of non-invasive cell-based therapy for retinal degeneration and associated vascular pathology.
The overall objective of this research proposal is to preserve vision and limit vascular pathology using non-invasive MSC therapy in rodent models for retinal degeneration. The MSCs have been widely used in both regenerative and degenerative medicine. The proposed research will be critical in determining whether systemic administration of MSCs offers a realistic likelihood of translation to the clinic for the treatment of retinal degeneration and ocular vascular pathology.
Role: PI

CS-RMI (start-up fund) Wang (PI)
06/01/2011-05/31/2015
Applying stem cell therapy for retinal degenerative disease

Department of Defense Wang (PI)
09/01/2012-08/31/2014
Non-invasive cell based therapy for traumatic optic neuropathy
Janssen Research & Development, LLC  Wang (PI)  08/01/13 - 07/31/15
Preclinical research with umbilical derived stem cell therapy for RCS rodent model for retinal degeneration.

The Simon and Beatrice Apple Stem cell fund  Wang (PI)  04/01/2014 - 03/21/2016
Develop personalized stem cells for treating AMD

Poster presented at ARVO meeting at Orlando, May 2014.

Systemic Administration of MSCs Preserves RGC Survival after Optic Nerve Crush

Wang S, Lin Y, Tai Y, Bakondi B, Girman S, Shen L, Svensson C and B Lu
Regenerative Medicine Institute, Department of Biomedical Science, Cedars-Sinai Medical Center, CA 90048

Abstract

Purpose: Traumatic optic neuropathy (TGN) results from trauma to optic nerves by head and eye injuries and is a major cause of vision loss. In a recent study, we demonstrated that subconjunctival administration of mesenchymal stem cells (MSCs) preserves retinal ganglion cell (RGC) survival after optic nerve crush (ONC) in mice. Therefore, the purpose of this study was to determine the effects of systemic administration of MSCs on RGC survival after ONC

Methods: Male C57BL/6J mice were assigned to one of the following groups: sham, ONC, MSC, or IONC. The sham group was subjected to anesthesia alone and the ONC group received unilateral ONC. MSC mice received intraperitoneal injections of MSCs 1 day before ONC and were sacrificed at 5, 7, and 14 days after ONC. IONC mice received intraperitoneal injections of MSCs 1 day before ONC and were sacrificed at 5, 7, and 14 days after ONC. For histological analysis, retinas were collected and fixed in 4% paraformaldehyde. The histological analysis was performed using Hoechst 33258 staining and TUNEL assay to detect apoptotic cells. The number of apoptotic cells in the ONC and IONC groups were compared using Student’s t-test. Data are presented as means ± standard deviation.

Results: There were no significant differences in cell survival or animal survival between the ONC and IONC groups. The ONC group had significantly less cell survival compared to the sham group at all time points. The ONC group had significantly more apoptotic cells compared to the sham group at all time points. The IONC group had significantly less cell survival compared to the sham group and the ONC group at all time points. The IONC group had significantly more apoptotic cells compared to the sham group and the ONC group at all time points. There were no significant differences in cell survival or animal survival between the MSC and IONC groups.

Conclusions: Systemic administration of allogeneic MSCs preserves RGC survival after optic nerve crush (ONC) in mice. These findings suggest that systemic administration of MSCs may be a potential therapeutic strategy for the treatment of traumatic optic neuropathy.