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PI: Jian Zuo, Ph.D.

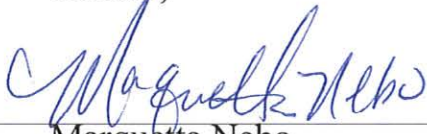
Organization: St. Jude Children's Research Hospital

ONR Award Number: N00014-12-1-0191

Award Title: Therapeutics for regeneration of fully functional auditory outer hair cells

Please find enclosed the Annual Technical Report of Dr. Jian Zuo. Should you have any questions or concerns, feel free to contact me at (901)595-2729.

Thanks,



Marquetta Nebo

Grant and Contract Administrator

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Organization: St. Jude Children's Research Hospital

ONR Award Number: N000141210191 (12PR04732-00)

Award Title: Therapeutics for regeneration of fully functional auditory outer hair cells

A. Scientific and Technical Objectives

Noise-induced hearing loss (NIHL) affects millions of navy servicemen even when the best protective devices are used. To address the Naval Global War on Noise (Naval Safety Center, 2007), we propose here, as a long-term goal, to develop therapeutics which would provide a framework for hearing restoration in naval servicemen who are suffering from NIHL.

NIHL is primarily caused by damage to sensory outer hair cells (OHCs) of the inner ear. Thanks to the ONR support, we have recently shown that SCs can be transdifferentiated into new HCs via overexpression of the transcription factor *Atoh1*. Although the new HCs appeared normal and survived for more than 3 months *in vivo*, new HCs formed in this manner were immature and lacked the expression of *Prestin*, a terminal differentiation marker of functional OHCs. These findings suggest that restoring OHCs requires more than *Atoh1* expression. Determining the unknown factors required to regenerate OHCs is a major obstacle to HC regeneration that must be overcome to restore hearing in service members suffering from NIHL.

We proposed to utilize a new mouse model (*Prestin*-YFP knockin) we have recently created to screen for candidate factors and small molecules to promote functional maturation of OHCs.

Aim 1. To identify combinations of genes which promote OHC maturation.

Aim 2. To identify small molecules which promote mature OHC formation.

Our new studies should provide candidate drugs as well as a list of previously unknown factors which will promote functional maturation of OHCs. These studies will also shed light on an unexplored area in basic hearing research.

B. Approach

- Develop *in vitro* assays from *Prestin*-YFP and *Atoh1*-Cre;tdTomato mouse inner ear stem cells/otospheres
- Develop and screen viral gene libraries that are likely involved in OHC maturation
- Screen libraries of small molecules (>8,835 bioactive compounds among more than 0.5 million compounds available at St. Jude)
- Secondary screens of *Atoh1*⁺ and *Prestin*⁺ OHCs

C. Accomplishments

1. We have successfully isolated otospheres from *Prestin*-YFP; *Atoh1*-Cre;Rosa-tdTomato mice;
2. We have successfully differentiated these otospheres into tdTomato and/or YFP expressing cells *in vitro*;

3. We have begun using Rho Kinase antagonists to overcome the cellular senescence inherent in otospheres.

D. Work Plan

Since the start of this award in March 2012, we have been focusing on the development of inner ear stem cell/otosphere assays appropriate for future high throughput screening purposes. We have made significant progress towards optimizing the conditions (i.e., otosphere senescence). We anticipate in the coming year we will fully develop and optimize the screening assays for high throughput screening.

E. Major Problems/Issues

We have encountered the issue of obtaining a large amount of YFP/tdTomato positive cells in vitro. We are exploring various methods (i.e., Rho kinase inhibitors) in culture to increase the yield and differentiation ability of otospheres.

F. Technology Transfer

Not applicable now.

G. Foreign Collaborations and Supported Foreign Nationals

We have hired a foreign national in our group as St. Jude employees using the ONR grant to work on this project.

- Dr. Shiyong Diao, a senior technician and a Chinese national, was hired in May, 2012 and has been working closely together with Dr. Brandon Walters on developing otosphere assays.

No collaboration with foreign institutes/individuals.