NJCSCR FINAL NARRATIVE REPORT: Neuroprotective function of Molecular Chaperones

1. Principal Investigator:

Dr. Alice Y. Liu, Department of Cell Biology and Neuroscience
Rutgers State University of New Jersey
604 Allison Road, Nelson Biology Labs.
Piscataway, New Jersey 08854-8082
Telephone (732)445-2730
Email: liu@biology.rutgers.edu

2. Name of Organization/Institution:

Rutgers State University of New Jersey

3. Grant Title

Neuroprotective function of molecular chaperones

4. Grant Number

05-3037-SCR-E-0

5. Grant Period Covered by the Report

9/30/2004 – 9/30/2009

6. Date of Submission of the Report

November 11th, 2009

BODY OF REPORT

The report should cover the following information in 2 - 5 pages, in addition to photographs, figures, charts, etc. Use language suitable for lay readers.

1. Original aims of the project.

Induction of the heat shock response (HSR) is an evolutionarily conserved defense mechanism against stress – a response mediated by the stress-dependent activation of heat shock factor 1 (HSF1) and results in the production of a family of HSP chaperones that perform manufacturing support and quality control roles in protein homeostasis, ensuring that newly synthesized proteins are complete, taken to the correct position within the cell's structure and correctly folded, and where there is a problem, the HSP chaperones will also direct a non-functional protein for degradation. It is generally recognized that the increased production of HSPs is essential for the maintenance of cell function and survival under stress. The goal of our research project is to harness the neuroprotective function of heat shock protein (HSP) chaperones to promote neuron survival after injury.

Our working hypothesis is that dysfunction of HSF1, and the decreased production of HSPs are sequential events that contribute to neuronal cell vulnerability in the injured spinal cord. We suggest that experimental means that boost the chaperoning function in cells - either by the forced expression of redox modifiers to prevent/reverse the inactivation of HSF1 and enhance the production of HSP molecular chaperones, or by the use of chemical chaperones - would enhance the survivability of cells under adverse conditions.

We note that the ability to minimize cell death cord is critical to functional recovery after spinal cord injury as studies showed that survival of a mere 10 percent of the neurons could allow the patient to maintain significant capabilities. Success in harnessing the neuroprotective function of HSP chaperones will spare neurons from irreversible injury and death after SCI and, in so doing, preserve functionality and improve the long term outlook of spinal cord injured patients.

2. Project successes.

We have made excellent progress in this research. The two major points of our research findings are:

1. Differentiation of neuroprogenitor cells from a stem cell-like state to one that resembles mature neurons is associated with a decreased induction of the heat shock response and an increased vulnerability to stress induced pathologies and death. This is consistent with observations that CNS and SC neurons have a limited capability to mount the cytoprotective HSR.

2. Agent(s) that boosts the HSR is cytoprotection. As an example, we showed that riluzole, the only FDA approved drug for the treatment of ALS, increases the amount of latent HSF1 for an amplified HSR and cytoprotection.

These are significant research findings given the consequential role of protein mis-folding in degenerative pathologies of neurons. Our observation that induction of the HSR is attenuated in the differentiated neuronal cell is consistent with the well known neuronal vulnerability to insults and injury. Our observation that agent/drug that up-regulates the induction of HSP chaperones is neuroprotection supports our contention that induction of HSP chaperone may be harnessed for therapeutics development in the treatment of SCI and other neurodegenerative conditions.

In the course of this research, we have developed and made available to interested investigators in the academic community our hsp70-firefly luciferase reporter gene constructs. This is a cell-based functional assay of HSF1 – it is most robust and allows for semi-high throughput screening of the effects of small molecules and treatment conditions on the regulation and function of HSF1. The polyclonal antibody that we generated against the recombinant human HSF1 protein and the experimental protocol that we have developed for the detection, resolution, and quantitation of redox conformers of HSF1 are available to the scientific community as well.

3. Project challenges.

Much of our research relied on biochemical analysis of cell extracts for the identification and quantitation of specific proteins involved in the induction of HSP chaperones. Because of the labor- and cost-intensive nature of the procurement and culturing of primary embryonic neurons – spinal cord neurons (SCN) included – it was difficult to routinely use primary neurons as our experimental material. Much of our studies – particular the regulation and function of HSR in neural differentiation – were done using tumor neuroprogenitor cells that can differentiate into neuron-like cells in a predictable manner. We will continue to strive to validate our research findings using both primary neurons and, ideally, in animal models of SCI.

4. Implications for future research and/or clinical treatment.

Given the breadth and depth of our understanding of the role of HSPs as a QC mechanism in protein homeostasis, it is perhaps surprising that this knowledge has not translated successfully to therapy for the prevention/treatment of a variety of protein mis-folding diseases – from SCI, neurodegeneration to diabetes. Perhaps, an important obstacle of the "translation" is that agents/conditions that induce the HSR are proteotoxic, and the induction of HSPs under such conditions represents a compensatory mechanism to rectify the perturbation of protein homeostasis. We

reasoned that drugs/small molecules that are not by themselves robust inducers of the HSR but which can enhance/amplify the effects of HSR inducers would have utility. For this research, we developed a semi-high throughput hsp70-luciferase reporter gene assay and successfully identified riluzole, the FDA-approved ALS drug, as an agent that boosted the HSR to promote cell survival under stress. Biochemical analysis showed that riluzole slows the turnover of HSF1 and increases the amount of HSF1 to support a more robust HSR for cytoprotection. Our result provides novel insight into the mechanism of turnover of HSF1, and identifies the degradation of HSF1 as a target for therapeutic intervention.

This research finding, published in PLoSOne in 2008 (Yang et al.), may have important implication in therapeutics development for the treatment of SCI. First, we note that the protective effect of riluzole is not limited to diseased motor neurons in ALS: riluzole has been shown to confer neuroprotection in spinal cord and cortical injury/ischemia, retard *huntingtin* aggregate formation in a cell free system and hippocampi organ culture, slow the progression of multiple sclerosis in human, and retard neuromuscular dysfunction in wobbler mouse motor neuron disease (see Yang et al., 2008 for citations). Second, The Reeve Foundation's North American Clinical Trials Network for the Treatment of Spinal Cord Injury (NACTN) is beginning its first clinical Phase I trial to test the safety of the drug riluzole in acute spinal cord injury (http://www.christopherreeve.org/site/c.ddJFKRNoFiG/b.5183975/k.1306/First_NACTN_Clinical_Trial_Set_for_Spring.htm). The trial which begins in 2009 is at 8 hospitals in the U.S. and Canada with riluzole administered <8 hours after injury. The PI for the riluzole trial is Michael Fehlings, MD, PhD, Professor of Neurosurgey at the U. Toronto. Studies from Dr. Fehlings' lab showed that riluzole protects nerve cells from the toxic cascade of events that occur in the minutes and hours after the initial trauma of SCI. We suggest that changes in the regulation and function of HSF1 likely contribute to the clinically efficacy of riluzole in the treatment of SCI and we are working towards validating our hypothesis.

5. Plans to continue this research, including applications submitted to other sources for ongoing support.

We are excited by the progress that we have been able to achieve. Our research strives to better understand the regulation and function of HSF1 and HSP chaperones. Given the fundamental importance of HSP chaperones as a protein QC mechanism that ensures the functioning and survival of cells and neurons under stress, we are confident that this research will have significant implication in our understanding of the pathogenesis as well as therapeutics development of SCI and other neurodegenerative conditions.

To support our research endeavor, we will continue to submit research grant application to national, state, and private agencies. Our work is currently supported by a Busch Memorial Research Grant.

6. List and include a copy of all publications emerging from this research, including those in preparation.

1. Oza J, Yang JX, Chen KY and **Liu, A. Y.-C.** (2008) Changes in the regulation of heat shock gene expression in neural differentiation. Cell Stress & Chaperones 13(1): 73-84.
2. Yang JX, Oza J, Bridges K, Chen KY, **Liu, A. Y.-C.** (2008) Neural differentiation and the attenuated heat shock response. Brain Research 1203: 39-50.
3. Yang JX, Bridges K, Chen KY, **Liu, A. Y.-C.** (2008) Riluzole increases the amount of latent HSF1 for an amplified heat shock response and cytoprotection. PLoSONE, 3(8):e2864. doi:10.1371/journal.pone.0002864.
4. Lu J, Gosslau A, **Liu A Y-C** and Chen KY (2008) PCR differential display-based identification of regulator of G protein signaling 10 as the target gene in human colon cancer cells induced by black tea polyphenol theaflavin monogallate. European Journal of Pharmacology 601: 66-72.
5. Gosslau A, Jao DL, Butler R, **Liu AY-C**, Chen KY. (2009) Thermal killing of human colon cancer cells is associated with the loss of eukaryotic initiation factor 5A. Journal of Cellular Physiology, 219:485-493.
6. Lee YK, Liu DJ, Lu J, Chen KY, and **Liu, AY-C.** (2009) Aberrant regulation and modification of heat shock factor 1 in senescent human diploid fibroblasts. J. Cellular Biochemistry. 106:267-278.

PUBLICATIONS AND PRESENTATIONS

All papers, presentations, chapters, and abstracts should mention that the research was supported by a grant from the New Jersey Commission on Spinal Cord Research. Copies must be sent to the NJCSCR office, even if you inadvertently forgotten to cite NJCSCR support.